The nature of robustness in development

H.F. Nijhout*

Summary

A trait is robust to a genetic or environmental variable if its variation is weakly correlated with variation in that variable. The source of robustness lies in the fact that the developmental processes that give rise to complex traits are nonlinear. A consequence of this nonlinearity is that not all genes are equally correlated with the trait whose ontogeny they control. Here we explore how developmental mechanisms determine and alter the correlation structure between genes and the traits that they control. A formula is developed by which the correlation of a gene or environmental variable with a trait can be calculated if the mechanism that gives rise to the trait is known. The nature of robustness and the ways in which robustness can evolve are discussed in the context of the problems that arise in the analysis of inherently nonlinear systems. ***BioEssays*** **24:553–563, 2002.**

Introduction

Terms such as robustness, canalization and developmental homeostasis are used to describe the stability of the phenotype in the presence of genetic and environmental variation. Although developmental biologists have amassed countless descriptions of developmental features that are canalized or robust to perturbation, we know little or nothing about the genetic or developmental mechanisms that lead to phenotypic stability. As a consequence, there is a certain ambiguity about the exact meaning of these terms. The same terms have been used to emphasize different aspects of phenotypic stability, and they have been used to refer to both a process and a result.[1] Because they do not refer to specific mechanisms, these terms are often used interchangeably and are assumed to be effectively synonymous.[2]

Not only do we not understand the mechanisms that lead to robustness, we have at present no means of quantifying robustness, or even an operational way of identifying robustness. If a developing organism is perturbed by mutations or physical stimuli, and the phenotype does not change, does this imply that the phenotype is robust to those perturbations? The lack of response could simply be due to the fact that those particular genetic or environmental factors do not affect any of the processes that lead to the phenotype in question. To be meaningful and measurable, robustness must refer to a specific trait and a specific perturbation, and the perturbation must be in a process or factor that is unambiguously part of the ontogeny or the function of the trait. No phenotype is equally robust, or sensitive, to all possible genetic or environmental perturbations. A quantitative measure of robustness should be able to rank the factors that contribute to the ontogeny of a trait according to the degree to which the trait is robust to their variation. A mechanistic understanding of robustness should be able to explain, and ideally be able to predict, this rank order.

To develop an operational definition of robustness, we begin with the premise that all traits are variable, that this variation is due to variation in the mechanisms that give rise to the trait, that this variation can have both genetic and environmental causes, and that this variation can be both natural and experimentally (or accidentally) induced. If variation of the trait is highly correlated with allelic variation in a specific gene, or with variation in a specific environmental factor, then the trait is not robust to that variation. By contrast, if variation in a trait is uncorrelated with variation in a particular genetic or environmental factor, then variation in that factor has no measurable effect on the trait and the trait can be said to be robust to variation in that factor (assuming, always, that the factor in question has a role in the ontogeny of the trait). The strength of the covariance of a trait with each of its various determinants is then a measure of the degree to which the trait is robust to variation in each of those determinants. This operational definition of robustness is independent of the mechanism by which robustness is realized; it encompasses both complex regulative development pathways, as well as simpler molecular homeostatic mechanisms.

Quantitative measures of the robustness of a trait to a variety of controlling factors could thus be obtained by measuring the covariances between the trait and each of the factors. If expressed as correlations (which are simply scaled covariances), then robustness can be expressed on a scale of 0 to 1. This measure would apply equally to genetic and environmental factors, as well as to variation introduced by experimental manipulation, all that is required are quantitative measures of the causes and their phenotypic effects. In the

Department of Biology, Duke University, Durham, NC 27708.
Funding agency: The National Science Foundation.
*Correspondence to: H.F. Nijhout, Department of Biology, Duke University, Durham, NC 27708.
DOI 10.1002/bies.10093
Published online in Wiley InterScience (www.interscience.wiley.com).

sections that follow I will explore how developmental mechanisms determine the correlation structure between genes and the traits they control. I will show how the correlation of a gene (or an environmental variable) with a trait can be predicted if the mechanism that gives rise to the trait is known and discuss the critical role that nonlinearities in developmental mechanisms play in the manifestation and evolution of robustness.

Robustness and penetrance

The problem of robustness (and its synonyms, like canalization) is related to the genetic problem of penetrance. Mutations with incomplete penetrance have a phenotypic effect in some individuals and not in others. Incomplete penetrance is usually assumed to be due to individual differences in the genetic background in which the gene acts, or to subtle differences in environment. Evidently, some genetic backgrounds (or environments) make a trait more robust to the mutation than others. Because incomplete penetrance is observed within a single breeding population, it implies that individual allelic differences in the genetic mechanisms of development can make traits more or less robust. Robustness can therefore be effected in two ways: by specially evolved mechanisms that somehow buffer the phenotype, and by genetic variation in existing mechanisms. The evolution of robustness can thus occur by the evolution of new genetic and physiological interactions in development, and also by selection on genetic variation in existing mechanisms.

The genetic background in which a particular gene acts is composed of all the other genes that in some way affect the properties of the trait in question. The profound effect of genetic background is best known from comparisons between genetically isolated populations. For instance, a particular gene knockout in mice can have a profound effect in one strain and be completely without detectable effect in a different strain.[3] Thus, strains of mice can differ greatly in their robustness to what by any measure would be a severe genetic insult. Likewise, oncogenic mutations are associated with disease in only a certain percentage of carriers, and this percentage can differ markedly in different populations.[4,5] Thus, robustness can be different in different populations, presumably in the same way that it can be different in different individuals within a population.

This kind of variable and context-dependent correlation between genetic and phenotypic variation in complex traits arises from nonlinearities in the genetic and developmental systems that generate the trait.[6] If the processes by which genes and other factors interact were linear and additive each causal factor of a complex trait would have a constant and consistent relationship to the trait. When these processes are nonlinear, however, the relation between genetic and phenotypic variation becomes context-dependent. Simply changing the values or frequencies of some alleles in a nonlinear system can cause other genes to change from being highly correlated with the trait to being uncorrelated, or vice versa.[6] Why this is so can be understood by means of a simple graphical example (Fig. 1). The vast majority of processes that control the ontogeny of a trait are nonlinear, so one would expect conditions like those outlined in Figure 1 to be common.

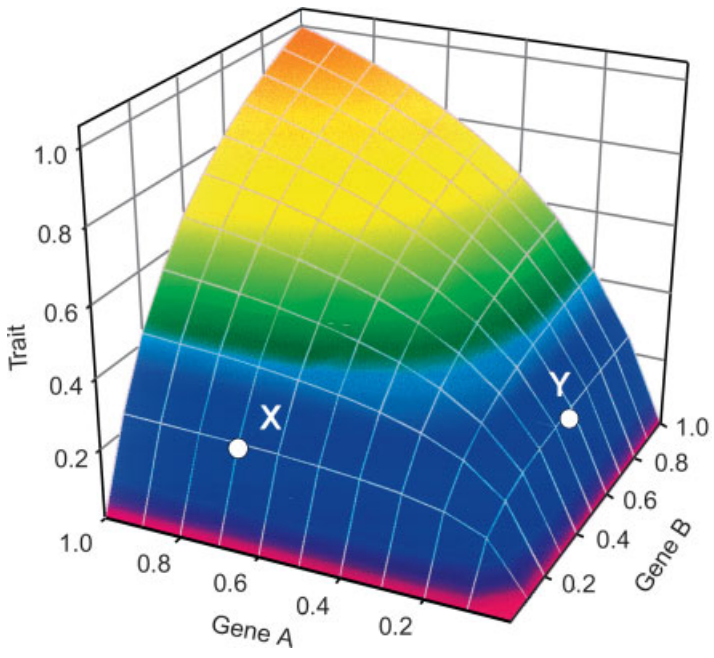


Figure 1. Phenotypic surface for a relatively simple complex trait, in this case a metabolic pathway consisting of a sequence of three enzymes, in which the product of one reaction serves as the substrate for the next one.[29] The trait (z axis) is flux through the pathway, and the independent variables are the activities of two of the three enzymes (x and y axes). All activities are scaled to unity. Points X and Y represent the mean genotypes (or mean genetic values with respect to each gene) of two populations that produce the same mean phenotype. Because of curvature of the surface, the correlations between genes A and B and the trait are different for the two populations. For populations centered around X, variation in gene B has a major effect on variation of the trait whereas a similar amount of variation in gene A is almost without effect. The converse is true for a population centered on Y. Thus for population X, gene B would be identified as a major gene whereas gene A might be detected as a modifier gene or quantitative trait locus (QTL), and vice versa for population Y. In terms of robustness, population X is robust to variation in gene A, and population Y is robust to variation in gene B.

Figure 1 also suggests a method by which the robustness of a trait to genetic variation can be evaluated without empirically measuring the covariances. The trait in this case is the flux through a biochemical pathway consisting of three enzymes. This flux is nonlinearly related to the activity of any one of the enzymes. It seems intuitive that the robustness of this trait to variation in any one of the factors (enzymes) that determine the trait is related in some way to the slope of the relationship between the trait and that factor. If the slope is great, then a given amount of variation in the factor will cause a larger amount of variation in the trait than if the slope is small. In the former case, the covariance between the factor and the trait would be greater than in the latter case, and trait would be considered to be less robust to variation in that factor. For instance, individuals at positions X and Y have the same phenotype, but for individuals at X, variation in enzyme A has little or no effect on the phenotype, but variation in enzyme B does. The opposite is true for an individual at Y. Thus for an individual at X, the phenotype would be robust to factors that alter the activity of enzyme A, but not to factors that alter B, and the reverse is true for individuals at Y. Again, the robustness of this trait is context dependent, and this context dependency arises from the nonlinearities in the relationship between the trait and the factors that determine the trait. Thus instead of empirically measuring the covariances or correlations between a trait and a determining factor, it appears that it might be possible to estimate the covariances (and robustness) from the mechanism by which various factors affect the phenotype.

Quantifying the causes of the phenotype

Although there exist a variety of statistical methods for quantifying the association between genes and complex traits,[7] none of them use information about the roles of genes in development to deduce the magnitudes of the associations. It is becoming increasingly clear, however, that the causal linkage between genetic variation and trait variation can be analyzed by means of mathematical simulations of real genetic mechanisms. Many genetic networks are now sufficiently well understood that it is possible to write equations that describe the interactions among their components. This has been done for the regulation of the cell cycle,[8,9] MAP kinase cascades,[10] Notch–Delta interaction,[11] diffusion-gradient-threshold mechanisms,[6,12] and segment polarity and *eve*-stripe expression in *Drosophila* embryonic development,[13–15] to name a few examples. Simulations, using mathematical models, make it possible to successfully predict the effect of mutations and experimental perturbation on a complex trait, as can indeed be done now with simulations of the cell cycle[8,9] and pattern formation in embryonic development.[13,14]

In a mathematical model of a genetic circuit, the activity of each gene is defined by an equation, typically a differential equation, that describes how the activity of the gene product is regulated. In a genetic circuit, for instance, one would write an equation for each gene that describes its activity (e.g. the rate of production of its product) as a function of various expression regulators. The system as a whole is represented by a set of coupled equations that describes the interaction of gene products with each other and with other components of the system, plus one or more equations that describe how the phenotype of interest is controlled by the relevant gene products. The equations that describe genetic regulatory interactions are typically nonlinear, and systems of such equations usually do not admit to a closed-form solution. Therefore, the phenotype cannot be expressed as a simple function of genotypic values. Instead, the phenotype must be calculated as a numerical solution of the system of coupled equations.

In a genetic regulatory circuit, we are actually interested in the proteins (transcription factors) that regulate the behavior of the system, not the genes themselves. Thus, although we may speak of genes, all the action is by proteins and it is the proteins that are the players when we model the effects of genes on development. Throughout this discussion, we assume that there is a simple relationship between genetic value (in the quantitative genetic sense) and the activity of the gene's products, or proteins. Gene activity represents the amount of protein being expressed, and this protein may be autonomously active or may require additional activation. A protein may also be dynamically activated and inactivated, as occurs in phosphorylation and dephosphorylation reactions of signaling molecules and cell cycle components. The expression level (amount of protein) is controlled exogenously, by transcriptional activators and inhibitors, while the activity parameter is a property of the protein molecule itself, whose value may be modified by specific factors in its physical environment (e.g. allosteric activators, phosphorylation, dimerization, temperature). Simulations of the ontogeny and function of complex traits can explicitly model dynamic changes in protein activity by incorporating the effects of regulators as additional functions.

In order to understand the properties of interactive systems it is helpful to visualize such systems of equations. This can be done by plotting the way that the phenotype varies with variation in the parameters of the system. The parameters are the rate constants that define the effect a given gene (or, rather, a gene product) has on the activation or inactivation of other genes, and on the structure or activity of other cellular components. The values of these parameters are the properties of a particular genotype. Allelic variation is reflected in a corresponding variation of parameter values. If the ontogeny or physiology of a trait can be described by a system of equations with n parameters, then it can be graphed as an n-dimensional surface in $(n+1)$-space, where each orthogonal axis represents a range of continuous variation in one of the parameters (Fig. 1 shows such a surface for two genes, in three-dimensional space). Visualization methods do not permit representation of more than three dimensions, so only

pair-wise visual analyses are possible. Figure 2 shows a selection of such pair-wise sections through the multidimensional surface of a system of six genes, and illustrates the diversity of surface shapes that can occur at different combinations of parameter values.

Many of the processes in development and physiology that lead to the complex phenotype are non-genetic. These include all the modes of long-distance communication, and all processes that depend on the physical structure or arrangement of cells, organelles, and macromolecules. A complete account of the ontogeny and function of a complex trait will have to include these physical factors as well. In a quantitative model of a trait, it is also possible to explicitly include the effects of environmental variables and experimental perturbation. For example, the effect of temperature can be modeled by the way that it affects rate constants, and the role of micronutrients or metabolic inhibitors can be included by the effect that they have on one or more specific processes in the ontogeny of function of a trait. In terms of a phenotypic surface, each environmental parameter can be represented as an additional orthogonal axis of variation. This type of depiction places environmental variation on equal footing with genetic variation, and allows one to explicitly study of the relative roles of genetic and environmental variation in phenotypic plasticity and reaction norms.

Individuals are represented by points on this multidimensional phenotypic surface (and populations as clouds of points). Mutations that *quantitatively* change the activities of gene products change the position of an individual on the surface, *but they do not alter the shape of the surface* (recall that the surface is merely a visual representation of a set of equations, and if the structure of the equations does not change then the surface does not change either). Changes in environmental conditions, likewise, move individuals on the surface (in this case parallel to an axis of environmental variation) but do not change the shape of the surface. The *shape* of the phenotypic surface can only be changed by changing the mechanism, that is, by adding or removing genes

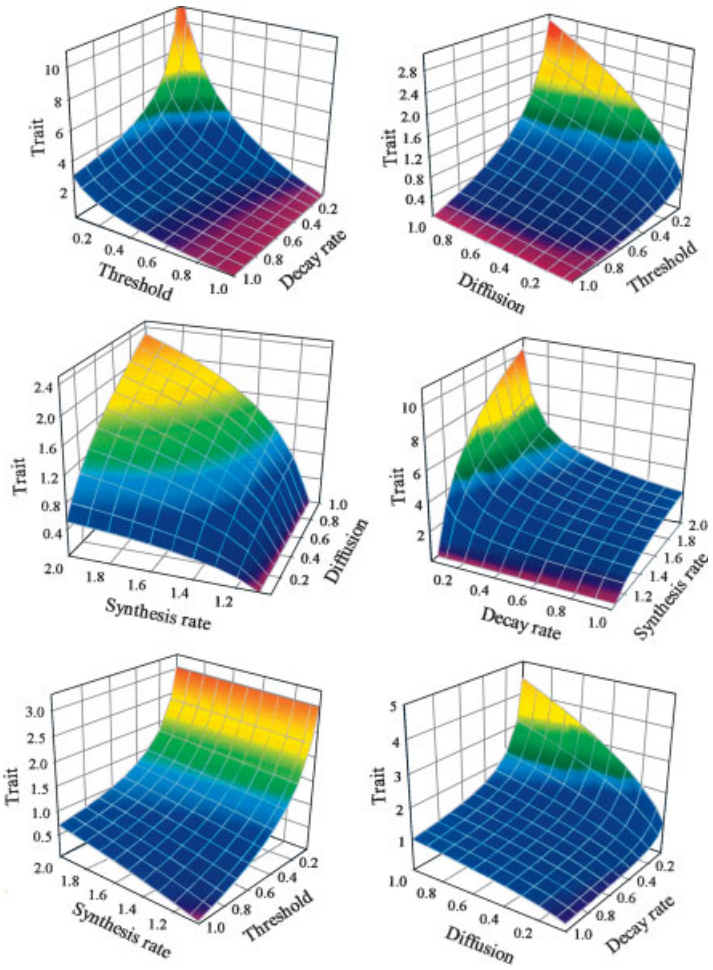


Figure 2. A selection of two-dimensional sections through the six-dimensional phenotypic surface that describes a complex trait produced by a diffusion-gradient-threshold mechanism.[6,12] The six parameters of this model are synthesis rate of a diffusing signal molecule at the source, decay rate of the signaling molecule, its diffusion coefficient, the threshold value, time at which the gradient is read, and background synthesis rate of the signal. All six parameters have a nonlinear relationship with the trait.[6] Although it is likely that the *value* of each parameter is determined by many genes, we assume, for simplicity, that the *variation* of each parameter is affected by only one gene. Hence, variation of the trait is governed by allelic variation of six genes. Genetic variation in this case is scaled to unity, except for variation in synthesis rate, which is scaled from 1.0 to 2.0. The full six-dimensional surface cannot be depicted, but can be easily obtained by computation.

(or environmental factors) from the system, or by mutations that qualitatively change the mechanism by which components of the system interact. Because the phenotypic surface is merely a graphical representation of the model, we can construct the shape of a phenotypic surface from the generative mechanism alone, without any knowledge of the genetic values of alleles, or of the genotypes of individuals. Those parameter values are necessary, however, to locate a particular individual on the surface.

The correlation between genetic and phenotypic variation

The derivative of the trait with respect to the genetic value of a gene product is a measure of the sensitivity of the trait to variation in that gene. In a phenotypic landscape, this is represented by the local slope in the direction of the axis that represents that gene. Thus in regions of the surface where the slope is small, a given amount of genetic variation will have a smaller effect on variation of the trait than in regions where the derivative is large (cf. Fig. 1). Because of nonlinearity, there will always be regions where the slopes relative to some determinants (genes or environmental variables) are relatively small, and these constitute regions where the trait would be considered to be robust, or canalized, with respect to variation in those determinants. Thus robustness to genetic variation would be revealed as a relatively small slope of the surface parallel to the axis that represents that gene. Our operational definition of robustness suggests that it might be useful to express the correlation or covariance of a trait with a particular causal factor in terms of the slopes of the phenotypic surface. In a system with *n* uncorrelated causal factors, the correlation of any one of them with the trait is given by

$$r_{f_1,\,t} = \sqrt{\frac{\beta_1^2\, Var(f_1)}{\sum_{i=1}^{n} \beta_i^2\, Var(f_i)}} \qquad (1)$$

where *f* is a causal factor (either a genetic or environmental parameter), and coefficient β is a regression coefficient (for the derivation of this equation see Appendix, which also gives the equivalent equation for correlated causes, e.g. linked genes). As we will see below, the regression coefficient β can be approximated by the slope of the phenotypic surface at the population mean genotype. Equation (1) can also be expressed in the following two forms, which are equivalent to and interchangeable with equation (1):

$$r_{f_1,\,t} = \frac{\beta_1 \sqrt{Var(f_1)\,Var(t)}}{\sum_{i=1}^{n} \beta_i^2\, Var(f_i)} \qquad Cov_{(f_1,\,t)} = \frac{\beta_1\, Var(f_1)\,Var(t)}{\sum_{i=1}^{n} \beta_i^2\, Var(f_i)}$$

The correlation (and the covariance) of any one factor with the trait can thus be obtained from the slopes of the phenotypic surface and the variances of the causal factors and trait. The form of equation (1) has the advantage that is does not require us to specify the variance of the trait, and that is uses the same kinds of terms in both numerator and denominator. The denominator shows that the correlation will change if the slope or the variance of any of the casual factors changes. It is important to note from equation (1) that the correlation is the effect of one factor divided by the sum of the effect of all factors. Since the square root is taken on the right side, this implies that the squared correlations (that is, the coefficients of determination) of all factors with the trait must sum to 1. The equation also shows that the correlation of a gene with a trait is a function of the *relative* slope, not the absolute slope of the phenotypic surface with respect to that gene.

The usefulness of this method for predicting the correlation between say, genetic variation and trait variation will depend on how well the nonlinear mechanistic model is approximated by the linear statistical model (Fig. 3), on how well the mathematical model simulates the form and function of the trait, and on how well we are able to estimate the parameter values. I will discuss these problems in turn below.

How nonlinear is a phenotypic surface?

The fit between a linear statistical model and a nonlinear mechanism will be fairly good if the range of variation of a

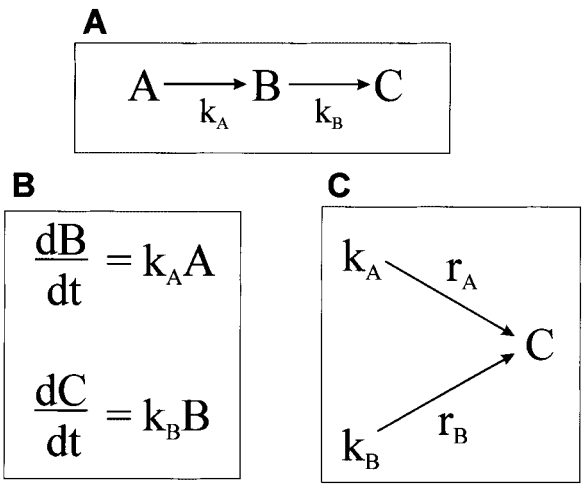


Figure 3. The relationship between a mechanistic model and a statistical model. **A:** A small linear genetic network in which gene A activates gene B, which in turn activates gene C. The rate of activation is given by the parameters k_A and k_B. These parameters constitute the genetic values of genes A and B and will be subject to allelic variation. **B:** A mechanistic model that could describe this relationship. **C:** If genes A and B are not linked, then their allelic variation is uncorrelated and the relevant path diagram that describes the correlation pattern of their joint effects on gene C. The corresponding statistical model is detailed in the appendix.

population is small relative to the nonlinearity. This is because over a small range of variation a curved surface will appear locally flat, and is this well-approximated by a linear model. A test of equation (1) for predicting correlations, using two different genetic mechanisms, each with two degrees of allelic variation, is shown in Fig. 4. In both cases, the fit between predicted and empirically determined correlations is remarkably good when the range of allelic variation is less than one order of magnitude, that is, when the physiological activities of alleles in a population differ from one another by a factor of less than 10. As expected, the linear model produces significant error when the range of allelic variation is very large.

We have at present no direct information about the range of functional allelic variation in natural populations. It is, however, possible to deduce that this range is typically small. Studies on genetic regulation have shown that the expression level of regulatory genes or the activity of their products, changes approximately two- to ten-fold in the process of regulation. For instance, the *bicoid* protein, which forms a gradient along the long axis of the *Drosophila* egg and controls the expression of early patterning genes, varies over a only two-fold range of concentration from the top to the bottom of the gradient,[16] and in that range regulates the expression of at least four genes with different thresholds. Other genes that control patterning of the *Drosophila* embryo, such as *Krüppel, knirps, giant* and *hunchback*, vary in concentration over a similar range as bicoid.[17] Epidermal growth factor receptor (EGFR) phosphorylation varies over a 4- to 6-fold range after stimulation with a ligand,[18] mitogen-activated protein kinase (MAPK) activity, varies over a three- to five-fold range in the feedback regulation of phosphatase synthesis,[19] and the cyclic expression of biological clock genes occurs over a three- to ten-fold range.[20] We can call this range of variation, between the generally inactive (subthreshold) and the generally active (above threshold) concentration of a regulatory gene product, the *dynamic range* of gene or protein activity.

The range of allelic values in a population is almost certainly smaller than the dynamic range of gene activity. The reason for this is that if the differences between the basal activities of the alleles in different individuals were of the same magnitude (or larger) than the dynamic range, it would be virtually impossible to achieve proper regulation of development and metabolism because extreme allelic values would effectively cause a gene to be permanently on or off in some individuals. Hence, if the dynamic range of gene activity is typically less than an order of magnitude, it is likely that the normal range of allelic values in a population is substantially less than an order of magnitude. Data on the effective range of allelic variation in human

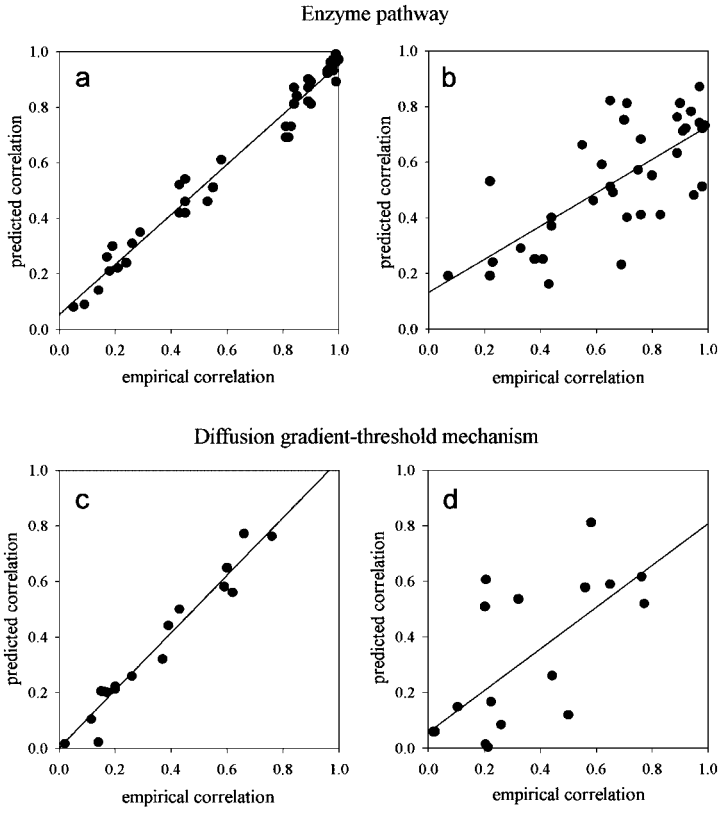


Figure 4. Relationships between empirically determined correlations and predictions for two different nonlinear systems. In each case, empirical correlations were determined by simulating several populations of 10,000 randomly mating diploid individuals with different randomly chosen allele frequencies, and different ranges of allelic values. The predicted correlations were obtained from equation (1) by taking the slopes at the population mean genotypic values. **a,b:** Results for the system described in Fig. 1, using two genes with three alleles each. **c,d:** results for the system shown in Fig. 2, using six genes with two alleles each. The left panels for each system illustrate predictability when allelic variation is less than one order of magnitude, the right panels when variation is between one and two orders of magnitude. In **a** the range of allelic values 3.37 (2.10) [mean (std dev)]-fold, giving $r^2 = 0.97$. In **b** the range of allelic values is 45.0 (23.1)-fold, giving $r^2 = 0.52$. In **c** the range of allelic values is 6.22 (2.46)-fold giving $r^2 = 0.95$. In **d** the range of allelic values is 60.6 (36.7)-fold giving $r^2 = 0.58$.

populations have recently been compiled by M. Rockman and G. A. Wray (Duke University; pers. comm.). These colleagues surveyed naturally occurring polymorphisms in the promoter regions of 107 human genes. Promoter polymorphisms result in different degrees of expression (transcription) of those genes. The studies that they surveyed typically monitored the effects of alternative promoters on the expression of a reporter gene in cultured cells. Their results show that 40% of the polymorphisms caused $<$ two-fold differences in expression; another 39% caused $<$ five-fold differences, and 16% caused $<$ ten-fold differences. Only 11% of the polymorphisms caused $>$ 10-fold differences in gene expression. It is believed that the assay methods used probably produce slight overestimates of the actual promoter activities (M. Rockman, pers. comm.). Overall, it appears that most (79%) regulatory polymorphisms produce less than five-fold variation in gene expression. It will be interesting to determine whether the polymorphisms that produce large differences in gene expression are associated with significant phenotypic variation.

Another way to estimate the degree of nonlinearity is to measure the non-additive components of genetic variance, using the methods of quantitative genetics.[21–23] In quantitative genetics, the genetic component of phenotypic variance (V_p) can be partitioned into additive (V_a), dominance (V_d), and epistatic (V_i) variances. The ratio V_a/V_p is the heritability of the trait, which is a measure of the resemblance between parents and offspring, and predicts the response of the trait to selection. The dominance and epistatic variances measure the effects of nonlinearities in the interactions among alleles and among genes, respectively, and are together referred to as the nonadditive components of the genetic variance. Dominance variance is typically much smaller than V_a, and V_i is smaller than V_d. Crnokrak and Roff[24] have surveyed a large number of studies that measured the relative size of V_d and found that for physiological and morphological traits the average ratio $V_d/(V_d + V_a)$ was 0.27 and 0.17, respectively. Epistatic variance is not often measured, and no good compilations are available, but it is generally substantially smaller than V_d.[23] In general, although the underlying mechanisms that lead to the phenotype may be highly epistatic, the empirical finding is that most of the genetic variance appears in the additive component, meaning that it can be explained by a linear regression model.[22,23]

There are two circumstances in which nonlinearity can become important. The first is when alleles differ greatly in their physiological effect (so that the variance is high), which could happen if there is a high mutation rate, or if null alleles are present in a population. The second is when populations that occur on different locations of a curved surface interbreed (e.g. populations represented by X and Y in Fig. 1). Even if the mean phenotype of each population is the same, and the local nonlinarity for each population is small, the extended range of allelic values and phenotypes in a population that receives an admixture of "foreign" alleles could become large relative to the curvature of the surface. In such cases, we would expect rapid selection to reduce the range of allelic variation until it is smaller than the dynamic range of genes.

Can we formulate adequate simulation models of the phenotype?

The ability to predict the correlation between genetic and phenotypic variation depends critically on how well the mathematical model that is used to construct the phenotypic surface simulates the trait and its variation. Because a model is essentially a mathematical description of empirical findings, we clearly need to have a substantial amount of factual information about a system before we are able to model it adequately. We need information about the basic mechanism that generates the trait of interest, but also information from perturbation experiments and mutations to ensure that aberrant behavior is also modeled accurately. Numerous examples of how to proceed with this kind of analysis can be found in the literature on metabolic systems.[25] There are at present a number of developmental systems for which we have sufficient structural and functional information for this level of simulation modeling,[8–15] and that number is increasing rapidly.

The critical issue that must be addressed in all types of modeling is how sensitive the behavior of the model is to *ceteris paribus* assumptions. In this case, how likely is it that a missing piece of the mechanism will drastically alter the shape of the phenotypic surface and thus change the predictions about the relative correlations of genes? Although it is not possible to determine a priori which genes or environmental parameters will have the greatest impact on a trait, it is not unreasonable to suspect that researchers will initially have focussed their studies on components to which the system happens to be most sensitive. If a system has been sufficiently well studied to permit accurate simulation, not only of the trait but also of the effects of known mutations and environmental perturbations, then it is likely that the most important factors have already been identified. In such cases, it is unlikely that new details will drastically alter the predicted correlation structure.

The allelic value problem

Studies on the correlation between genes and traits, and on the effects of mutation and selection, however, require us to place individuals and populations on the surface, and that requires specification of the allelic values of individuals, the range of functional allelic variation in populations, and the environmental parameters of the model. The values of the environmental parameters will typically be relatively easy to specify, but our knowledge about the actual physiological values of alleles, and the range of those values in natural populations, is quite limited. This is what we might call the allelic value problem. Although we know the connectivities in

many genetic circuits, we often have little direct quantitative information about the biochemical or physiological "activities" of gene products in situ (although some of these values may be deduced computationally[13,15]. Even less is known about the diversity of functionally different alleles in a population, though this is likely to change rapidly in the near future with advances in genomic and proteomic technology.

In the absence of specific information, it is nevertheless useful to ask how accurately the allelic "values" of an individual need to be specified. Recent simulation studies on realistic developmental and genetic systems suggest that they can be remarkably robust to parameter variation: small to moderate changes in parameter values have little effect on the outcome of the process. For instance, Bodnar,[26] has simulated the genetic networks for early segment specification in the *Drosophila* embryo, using Boolean rules to effect switching of genes. He found that by specifying gene activity with only four values (zero, low, medium, and high), and by allowing time to progress in discrete steps of one nuclear cycle, he could get quite exact simulations of the spatial and temporal expression patterns of the more than twenty genes that are involved in the regulation of this system. A different approach to simulating the segment polarity network in the *Drosophila* embryo, using a continuum model of coupled differential equations, likewise revealed that this system is remarkably robust and can tolerate as much as a ten-fold variation in the values of many of its parameters without substantial changes in the output of the model.[15] A sensitivity analysis of ten rate constants of the cell cycle mechanism simulated by Novak et al.[27] (their Figure 9), revealed that seven of these can tolerate a two- to three-fold variation around the parameter values used by the authors, one can tolerate a slightly less than two-fold variation, and the system is fairly sensitive to variation in the remaining two (Nijhout, unpublished). These observations suggest that complex developmental networks may be relatively insensitive to variation in all but a few parameters. The degree of sensitivity to parameter variation will of course be different in different systems and, within a system, it will depend on the gene in question, and on its interactions with other casual factors. The relative insensitivity to parameter variation suggests that these systems may be tolerant to moderate accuracy in specification of their parameters.

The nature of robustness

In terms of a phenotypic surface, a lack of sensitivity to variation in a genetic parameter implies that the slope of the surface parallel to the axis that specifies that parameter is small (in terms of the model equations, it implies that the first derivative of the genetic or factor values with respect to the trait is small).[28–30] As alluded to above, it is the general property of nonlinear systems that the slopes vary with parameter value, and that, therefore, there will always be regions in parameter space where a set of genetic or environmental variables has relatively small slopes. In those regions, the system will appear to be robust (or canalized) with respect to those particular (genetic or environmental) parameters, and their variation will have relatively little effect on variation of the trait. There are, therefore, two ways in which robustness can evolve. The first is by mutations and selection on quantitative variation in gene activity that move a population to a new region of the phenotypic surface where the slopes of the relevant genes are relatively smaller. The second way is by changing the mechanism that generates the trait, for instance by adding a feedback loop or incorporating novel interactions in a genetic circuit. This could be done by mutations in the regulatory region of a gene that change the kinds of transcriptional regulators that it interacts with. Such changes will change the shape of the phenotypic surface and could make the *relative* slopes of some genes smaller without actually requiring mutational changes in, or selection on, those genes.

Equation (1) shows that the correlation of any one factor with the trait depends on all the other factors in the system. The more factors there are, the smaller the correlation of any one of them will be, *on the average*. Therefore, one would expect that for very large systems the correlations would, on the average, be very small. In nonlinear systems, the correlations will, however, not all be the same; some will be larger than others, and some may be quite large. It is interesting to note that the number of factors that can have large correlations with the trait is limited by the summation property: the coefficients of determination must sum to 1. The summation property suggests that only a few factors can be highly correlated with the trait, and be uncorrelated with each other. Uncorrelated genetic causes arise if genes are on different chromosomes and segregate independently. Linkage and gametic phase disequilibrium cause genes to be correlated with each other to various degrees, and genes that are highly correlated with each other will have similar correlations with a trait (the correlations can be calculated from equation (A4.1) if the degree of linkage is known). Likewise, environmental factors whose variation is highly correlated will also have similar correlations with the trait.

The summation property puts severe limitations on the number of genes and environmental factors whose independent effects on a trait can be detected. For instance, if our data do not allow us to detect correlations smaller than 0.3 (as significantly different from zero), then we will be able to detect at most 11 factors ($11 \times 0.3^2 = 1$), assuming all are equally correlated with the trait. If two of these factors have a higher correlation with the trait, say 0.6, then it will be possible to detect at most three additional ones (because $2 \times 0.6^2 + 3 \times 0.3^2 = 1$). This does not mean that there are no other genetic or environmental factors that are important in the ontogeny of the trait. It simply means that the effect of their *variation* cannot be detected. The trait is effectively robust to variation in those factors.

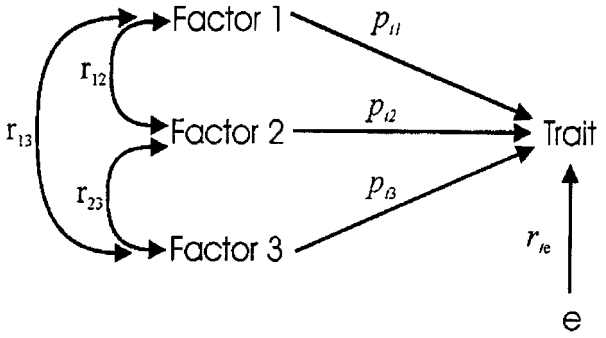
Robustness can therefore be both an evolved as well as a natural property of complex systems. Large and interactive developmental systems will be naturally robust to variation in many of their components, but it is also likely that such systems will be quite sensitive to variation in a small subset of their component processes. If such lack of robustness happens to be to factors that are also quite variable, then there will be selection to either reduce that variation, or selection on other factors or mechanisms that reduce the correlation.

Acknowledgments

I am indebted to Mark Rausher for insightful discussions and constructive critiques of early versions of this work. I would also like to thank Jeff Marcus, John Mercer, Armin Moczek, Tomalei Vess, Andy Yang, and several anonymous reviewers for helpful comments that (I hope) improved this paper.

Appendix

The value of the correlation between trait variation and variation in the determinants of a trait can be found with the help of path analysis.[22,30,31] Here the term "factor" refers to any factor that contributes to or affects the ontogeny or function of a trait. These can be genes (in which case variation refers to allelic variation in a population), environmental variables such a temperature or nutrition (which could be micronutrients such a folate, or bulk nutrients that affect overall growth rate), and experimental interventions, that affect different members of a population differently. Typically in path analysis, the values of the correlations are determined empirically. The object here is to deduce their values from the effect each factor has on the trait. We would like to express the correlation in terms of the slopes of the phenotypic surface and the distribution of individuals on the surface. If variation in a trait is controlled by variation in n factors that are correlated with each other (e.g. due to linkage or gametic phase disequilibrium), the following path diagram applies (illustrating the case for $n = 3$):



where r is a correlation coefficient and p is a path coefficient (a path coefficient is a standardized partial regression coefficient). *A path diagram is not a depiction of a causal network, but a description of the causes of variation in a system. If there is no variation in a factor (a monomorphic gene or a constant environmental factor) then it has no effect on variation of the trait and is not included in a path diagram, even though that factor may well be critical in the ontogeny or function of the trait.* The equation that describes the relationship shown above is derived from multiple regression and path analysis:

$$\sum_{i=1}^{n} p_{ti}^2 + 2\sum_{i=1}^{n}\sum_{j>i}^{n} p_{ti}p_{tj}r_{ij} + r_{te}^2 = 1 \qquad \text{(A1)}$$

This is the *equation of complete determination*. For its derivation, see Lynch and Walsh.[22] Here r is a correlation coefficient and p is a path coefficient, i and j are factors, and t is the trait. In the derivation below, we temporarily ignore the error term r_{te}^2, which will be added later as a separate term. Replacing the right-hand side of equation (A1) with r^2/r^2 and rearranging, the correlation of any one factor (f_1) with a trait (t) can be written as

$$r_{f_1,t}^2 = \frac{r_{f_1,t}^2}{\sum_{i=1}^{n} p_{ti}^2 + 2\sum_{i=1}^{n}\sum_{j>i}^{n} p_{ti}\, p_{tj}\, r_{ij}}. \qquad \text{(A2)}$$

Using

$$r_{f,\,t} = \frac{Cov(f,t)}{\sqrt{Var(f)\ Var(t)}},$$

and the fact that the slope b of the least squares regression of trait variation on factor variation in a population is given by:

$$b = \frac{Cov(f,t)}{Var(f)}, \quad \text{so } Cov(f,t) = b\ Var(f),$$

the numerator of (A2) can be written as:

$$\frac{b_1^2\ Var(f_1)}{Var(t)} \qquad \text{(A3.1)}$$

If there are correlations among the factors then b must be replaced by the partial regression coefficient β. The relationship between b and β is

$$b_1 = \beta_1 + \sum_{i=2}^{n} \beta_i \frac{Cov(f_1, f_i)}{Var(f_1)},$$

so the numerator of (A2) becomes

$$\left[\beta_1 + \sum_{i=2}^{n} \beta_i \frac{Cov(f_1, f_i)}{Var(f_1)}\right]^2 \frac{Var(f_1)}{Var(t)}.$$

For the first summation term in the denominator of (A2), we note that a path coefficient is a standardized partial regression coefficient, and is defined as:

$$p_{it} = \beta_i \frac{\sqrt{Var(f_i)}}{\sqrt{Var(t)}}.$$

The first summation term can therefore be rewritten as:

$$\sum_{i=1}^{n} \frac{\beta_i^2\, Var(f_i)}{Var(t)}. \qquad \text{(A3.3)}$$

Likewise, the second summation term of (A2) can be rewritten as:

$$2\sum_{i=1}^{n}\sum_{j>i}^{n} \beta_i \frac{\sqrt{Var(f_i)}}{\sqrt{Var(t)}} \beta_j \frac{\sqrt{Var(f_j)}}{\sqrt{Var(t)}} \frac{Cov(f_i f_j)}{\sqrt{Var(f_i)Var(f_j)}},$$

which simplifies to

$$2\sum_{i=1}^{n}\sum_{j>i}^{n} \frac{\beta_i \beta_j Cov(f_i f_j)}{Var(t)}. \qquad \text{(A3.4)}$$

Substituting all this into (A2), multiplying numerator and denominator by Var(t), and taking the square roots, the correlation becomes:

$$r_{f_1,\,t} = \sqrt{\frac{\beta_1^2 Var(f_1) + \left[\sum_{i=2}^{n} \beta_i Cov(f_1, f_i)\right]^2}{\sum_{i=1}^{n} \beta_i^2 Var(f_i) + 2\sum_{i=1}^{n}\sum_{j>1}^{n} \beta_i \beta_j Cov(f_i, f_j)}}. \qquad \text{(A4.1)}$$

The correlation of any one factor with the trait is thus determined by the regressions of factor on trait values, the variances of the factor values, and the covariances among the factors. This expression is cumbersome, to say the least. However, if the genes are uncorrelated with each other and with the environmental variables, as in the cases analyzed here, the covariances are zero and the summation terms containing them drop out, so the correlations become

$$r_{f_1,\,t} = \sqrt{\frac{\beta_1^2\, Var(f_1)}{\sum_{i=1}^{n} \beta_i^2\, Var(f_i)}}, \qquad \text{(A4.2)}$$

Most natural populations are not in linkage disequilibrium, so equation (A4.2) is likely to be broadly applicable for genes that are not physically linked.

The error term

$$r_{e,t}^2 = \frac{Cov(e,t)^2}{Var(e)Var(t)}$$

is derived in the same way. It measures the portion of the correlation that is due to uncontrolled factors and is to be added to the denominator of (A4.1) and (A4.2).

Equations (A4.1) and (A4.2) can be expressed in various alternative forms. If 1 is expressed as r/r in equation (A2), the derivation leads to the following equivalent of (A4.2)

$$r_{f_1,\,t} = \frac{\beta_1 \sqrt{Var(f_1)Var(t)}}{\sum_{i=1}^{n} \beta_i^2 Var(f_i)}.$$

The relationship between factor and trait variation can also be expressed as a covariance by the same procedure and yields:

$$Cov(f_1, t) = \frac{\beta_1 Var(f_1) Var(t)}{\sum_{i=1}^{n} \beta_i^2\, Var(f_i)}.$$

References

1. Nijhout HF, Davidowitz G. Developmental perspectives on phenotypic plasticity, canalization, and fluctuating asymmetry. In: Polak M, ed.; Developmental Instability: Causes and Consequences. Oxford University Press, 2002. (in press).
2. Gerhart J, Kirschner M. Cells, Embryos, and Evolution. Malden, Massachusetts: Blackwell Science. 1997.
3. Dietrich WF, Lander ES, Smith JS, Moser AR, Gould KA, Luongo C, Borenstein N, Dove W. Genetic identification of Mom-1, a major modifying locus affecting *Min*-induced intestinal neoplasia in the mouse. Cell 1993;75:631–639.
4. Khoury MJ, Beatty TH, Cohen BH. Fundamentals of Genetic Epidemiology. New York: Oxford Univ. Press, 1993.
5. Weiss KM. Genetic Variation and Human Disease: Principles and Evolutionary Approaches. New York: Cambridge Univ. Press, 1993.
6. Nijhout HF, Paulsen SM. Developmental models and polygenic characters. Amer Nat 1997;149:394–405.
7. Haines JL, Pericak-Vance MA. Approaches to Gene Mapping in Complex Human Diseases. New York: Wiley-Liss, 1998.
8. Novak B, Tyson JJ. Numerical analysis of a comprehensive model of M-phase control in *Xenopus* oocyte extracts and intact embryos. J Cell Sci 1993;106:1153–1168.
9. Novak B, Tyson JJ. Quantitative analysis of a molecular model of mitotic control in fission yeast. J Theoret Biol 1995;173:283–305.
10. Huang C-HF, Ferrell JE. Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc Natl Acad Sci 1996;93:10078–10083.
11. Collier JR, Monk NAM, Maini PK, Lewis JH. Pattern formation by lateral inhibition with feedback: a mathematical model of Delta-Notch intercellular signaling. J Theoret Biol 1996;183:429–446.
12. Klingenberg CP, Nijhout HF. Genetics of fluctuating asymmetry: a developmental model of developmental instability. Evolution 1999;53:358–375.
13. Reinitz J, Sharp DH. Mechanisms of *eve*-stripe formation. Mech Dev 1995; 49:133–158.
14. Sharp DH, Reinitz J. Prediction of mutant expression patterns using gene circuits. BioSystems 1998;47:79–90.
15. Von Dassow G, Meir E, Munro E, Odell GM. The segment polarity network is a robust developmental module. Nature 2000;406:188–192.
16. Driever W, Nusslein Volhard C. The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. Cell 1988;54:95–104.
17. Reinitz J, Mjolsness E, Sharp DH. Model for cooperative control of positional information in *Drosophila* by *bicoid* and maternal *hunchback*. J Exp Zool 1995;271:47–56.
18. Haugh JM, Schooler K, Wells A, Wiley HS, Lauffenburger DA. Effect of epidermal growth factor receptor internalization on regulation of the phospholipase C-γ1 signaling pathway. J Biol Chem 1999;274:8958–8965.
19. Brondello J-M, Brunet A, Pouysségur J, McKenzie FR. The dual specificity mitogen-activated protein kinase phosphatase-1 and -2 are induced by the p42/p44^{MAPK} cascade. J Biol Chem 1997;272:1368–1376.
20. Yagita K, Tamanini F, van der Horst GTJ, Okamura H. Molecular mechanisms of the biological clock in cultured fibroblasts. Science 2001;292: 278–281.
21. Roff DA. Evolutionary Quantitative Genetics. New York: Chapman and Hall. 1997.
22. Lynch M, Walsh B. Genetics and Analysis of Quantitative Traits. Sunderland: Sinauer, 1998.
23. Falconer DS, Mackay TFC. An Introduction to Quantitative Genetics. Essex: Addison Wesley Longman, 1996.
24. Crnokrak P, Roff DA. Dominance variance: associations with selection and fitness. Heredity 1996;75:530–540.

25. Voit EO. Computational Analysis of Biochemical Systems. Cambridge: Cambridge Univ. Press, 2000.
26. Bodnar JW. Programming the *Drosophila* embryo. J Theoret Biol 1997; 188:391–445.
27. Novak B, Csikasz-Nagy A, Gyorffy B, Nasmyth K, Tyson JJ. Model scenarios for evolution of the eukaryotic cell cycle. Phil Trans Roy Soc London 1998;B 353:2063–2067.
28. Rice SH. The evolution of canalization and the breaking of Von Baer's laws: modeling the evolution of development with epistasis. Evolution 1998;52:647–656.
29. Kacser H, Burns JA. The molecular basis of dominance. Genetics 1981;97:639–666.
30. Li CC. Path Analysis—A Primer. Pacific Grove: Boxwood Press, 1975.
31. Sokal RR, Rohlf FJ. Biometry. New York: Freeman, 1995.