CHAPTER 3

Dominance, Nonlinear Developmental Mapping and Developmental Stability

Christian Peter Klingenberg

Abstract

Developmental stability is the ability of organisms to buffer against the random variation that arises spontaneously as a consequence of stochastic variation in the cellular processes that are involved in the development of morphological structures. Its converse, developmental instability, is the imprecision that leads to morphological variability even when genetic and environmental conditions are kept constant, and can be conveniently measured as the random left-right differences of bilaterally symmetric organisms. This chapter demonstrates that the genetic control of developmental stability is intimately connected with nonadditive genetic variation of the morphological traits of interest. Dominance and epistasis have also been shown by empirical studies to play an important role in the genetic architecture of developmental stability. A brief review of some mechanisms that generate stochastic variation in gene expression suggests that nonadditive genetic variation is also an important factor for the origin of developmental noise.

Introduction

The molecular and cellular processes that constitute development are inherently variable, yet they contribute to the reliable assembly of intricately organized body plans. The mechanisms that achieve this reliability at the level of the phenotype are referred to as developmental stability. The nature of these mechanisms is not well understood, and research going beyond purely phenomenological studies has only started relatively recently.[1] Developmental stability is subset of a broader class of developmental buffering phenomena, which also includes canalization against genetic and environmental effects.[2,3] It has been contentious whether different mechanisms are involved in developmental stability and canalization[4,5] or whether both are due to the effects of the same system on different kinds of variation.[6,7]

It is also an open question to what extent developmental buffering results from the action of special mechanisms or from robustness that is a consequence of the setup of developmental mechanisms. The most prominent case in favor of special mechanisms is the case of the Hsp90 chaperone protein, where the protein has a clear function in the maintenance of cellular function and inhibition of its action results in increased phenotypic variation and higher incidences of overt anomalies of development.[8-10] It is unclear, however, how far this example can be generalized, as a variety of developmental systems have been shown to be inherently robust against intrinsic and extrinsic perturbations by virtue of the way their components interact.[11,12] It is not evident whether buffering is a result of special mechanisms that have evolved as an

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adaptation for this function[13] or whether it arose as an automatic attribute of developmental systems, although buffering is clearly evolvable to a certain degree. This question is a parallel to the debate on whether dominance is an evolved property of genes or an automatic result of the biochemical and physiological function of genetic systems.[14-18]

In this chapter, I will primarily explore those origins of developmental buffering that result from the general setup of developmental systems, because they are directly linked to the issue of dominance and haploinsufficiency. Because this argument relies primarily on the nonlinear relations between inputs and outputs of developmental systems, which is a very widespread property of biochemical and developmental processes, it is likely that the conclusions apply to a broad range of organisms and developmental contexts. I also briefly review the literature on the molecular origins and implications of developmental noise, which may itself relate to dominance and haploinsufficiency.

Developmental Stability and its Measurement

Developmental stability is the ability of developmental processes to resist fluctuations of the system and produce a consistent phenotype according to the genotype and environment of the organism.[1] This degree of resistance against possible perturbations is inherently difficult to measure, but it is easier to quantify its opposite, developmental instability, which is the imprecision of a developmental system and its morphological end products. It can be quantified by the amount of variation among phenotypes that would be produced by the same developmental process run repeatedly under identical genetic and environmental conditions, or in a situation close to this ideal.

In bilaterally symmetric organisms or parts, fluctuating asymmetry,[1,19,20] the random differences between left and right sides, offers an easy means to study developmental instability. Both sides share the same genome (barring somatic mutations) and usually develop under nearly identical environmental conditions, and therefore the variation of asymmetry around its average is due to random fluctuations of developmental processes, and can be used as a measure of developmental instability.[1] This measure of developmental instability is a composite of the opposing effects of developmental noise and the system's capacity to buffer against it.

Similar reasoning has been applied at the cellular scale to quantify the relative contributions to stochastic variation of gene expression that are intrinsic and extrinsic to individual genes.[21,22] These components of variation can be separated by studying the expression of two identical genes in the same cell, for instance, two constructs with different varieties of a reporter gene such as green fluorescent protein.[22] Random variation that is intrinsic to a gene can be isolated from the contrast between the expression levels of the two genes, whereas the extrinsic component of noise can be estimated from the variation that is correlated between the two copies.[21,22] The logic of the comparison of the behavior of two genes in a cell corresponds to the comparison between the left and right sides of an organism in studies of fluctuating asymmetry.[1]

Developmental Mapping and Nonadditive Genetic Effects

Development has often been characterized by the relationship between the genotype and phenotype, described mathematically as some type of mapping function.[23,24] This approach can be extended to a consideration of both genetic and environmental contributions to the phenotype through a more general form of developmental mapping in which the phenotype is expressed as a function of some developmental parameter that is controlled jointly by genetic and other factors. The basis of this approach is a developmental or biochemical model, such as a diffusion-threshold process,[12,25,26] models of flux in a pathway of several enzymes,[14] or of the formation of molecular complexes.[27] For most developmental systems, these relationships are

markedly nonlinear, and this nonlinearity is of critical importance for the dynamical behavior of these systems.

At the core of this approach is a dissection of the relationship between genetic and other inputs into the developmental system and the morphological output into two distinct layers or phenotypes: the first level is a developmental phenotype, which can be conceptualized as the parameters of a developmental model, and the second level is the morphological phenotype that results from the model with those parameter settings. The benefit of introducing an intermediate developmental phenotype between the genetic factors and the morphological outcome is that the genetic control of the developmental phenotype has a relatively simple basis, even in complex developmental models. For instance, if the developmental parameter corresponds to the dose of a protein, it is possible to assume an additive genetic basis. The second layer of the model considers these parameters jointly with the nongenetic factors that influence development, and thereby takes into account the epigenetic and gene-by-environment interactions between different components of the developmental model. The genetic behavior of the morphological phenotype can then be examined by combining the information about the known genotypes and the morphological output of the system.[12,25]

In a developmental process that consists of several sequential steps, the aggregate result of all effects will be passed from one step to the next without reference to their cause. Regardless of whether a given change in the activity of, say, a molecular signal is caused by a specific allele, by an environmental influence or even by a random fluctuation of the system, it will have the same effect on the subsequent step where the signal is received. This sort of process therefore provides opportunities for interactions among the effects of different origins.

The nonlinearity of developmental mapping functions is critical for the origin of nonadditive genetic effects in such systems.[12,14,25,26] Dominance at a locus, or more precisely, dominance between two alleles of a locus, is defined as a deviation of the phenotypic value of the heterozygote for the two alleles from the average of the phenotypic values for the two homozygotes.[28,29] If each allele corresponds to a particular level of activity for a component in the developmental system, such as the transcription rate for the gene or the metabolic rate of an enzyme, the phenotypic values can be graphed as a function of this activity level (Fig. 1). In this graph, dominance corresponds to a deviation of the graph from a straight line, which corresponds to completely additive gene effects. Dominance can be quantified by the degree to which the phenotypic value of the heterozygote is above or below the value midway between those for the two homozygotes.[28] In other words, the degree of dominance for two alleles is a consequence of the extent to which the developmental mapping function is nonlinear in the range defined by those alleles.

There are different kinds of such curves, and accordingly, there are different kinds of dominance. Dominance can be positive or negative, depending on whether the phenotypic value for the heterozygote is greater or less than the value intermediate between the two homozygotes, or correspondingly, whether the developmental mapping function is convex or concave upward (Fig. 1). If the magnitude of the dominance effect exceeds the additive (linear) effect, that is, if the phenotypic value for the heterozygote is greater or less than the values for both homozygotes, there is overdominance or underdominance, corresponding to $\cap$-shaped or $\cup$-shaped mapping functions, respectively (Fig. 2). Moreover, even for monotonically increasing or decreasing functions, the shape of the mapping function can vary, which may affect the dominance patterns at the level of the phenotype. Some of these curves are steep at low values and increase toward an asymptote, such as the biochemical model of dominance based on enzyme kinetics by Kacser and Burns,[14] whereas models including cooperative binding of multiple molecules produce sigmoid curves.[27]

Depending on the location of the inflection point of a sigmoid mapping function relative to the position of the genotypes at the locus of interest, this form of curve can be the basis for

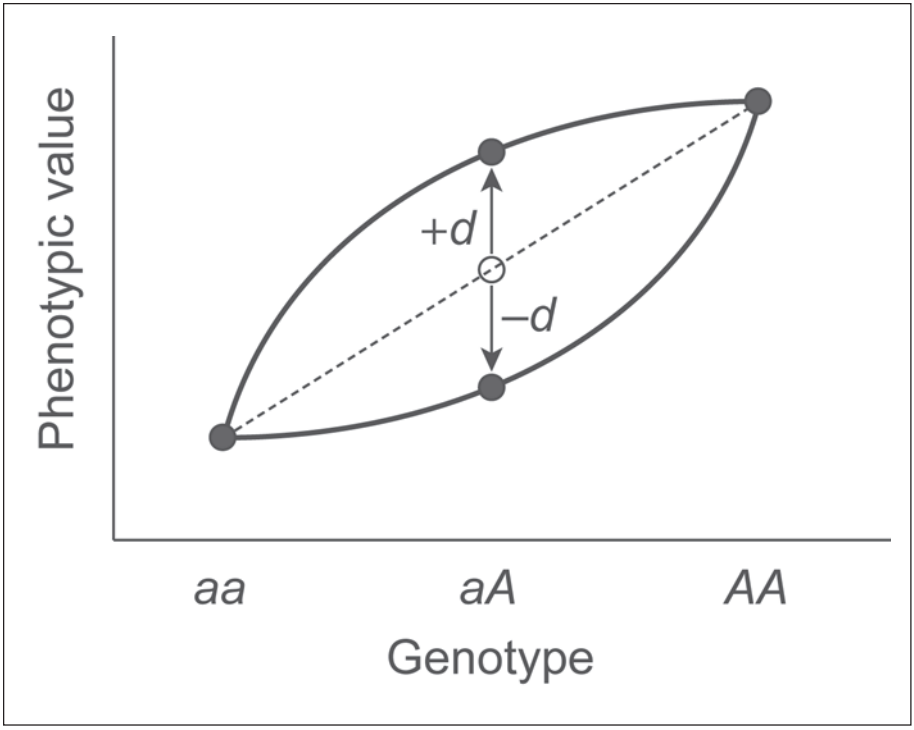

Figure 1. Nonlinear developmental mapping functions and dominance. Two developmental mapping functions are represented by the bold lines. Because they are curved, there is a difference between the phenotypic values and the midpoint of the phenotypic values for the two homozygotes. This deviation can increase or decrease the phenotypic value, corresponding to positive dominance (+*d*) or negative dominance (–*d*). The dashed line represents a linear developmental mapping function and the empty circle corresponds to the phenotypic value for completely additive effects of the gene.

haploinsufficiency (Fig. 3).[27] For mapping functions that increase in a sigmoidal fashion, haploinsuffieciency occurs if the inflection point is to the right of the developmental value of the heterozygote. In general, haploinsufficiency occurs for mapping functions where the allele with the lower level of activity is dominant, so that the heterozygote phenotype is similar to the "loss-of-function" phenotype. If the value for the "wild-type" phenotype is greater than the value for the "loss-of-function" allele, then any mapping function that is concave upwards will produce haploinsuffiency (e.g., Figs. 1, 3). Some caution is necessary, however, as pointed out by Veitia,[27] because it is not possible to distinguish haploinsufficiency from dominant negative effects based only on the phenotypes. Both haploinsufficiency and dominant negative effects are manifest as phenotypic changes in heterozygous condition, and therefore are dominant, but they differ in the manner in which they come about. Haploinsufficiency is the effect of reduced gene activity by mutation, where a single dose of the gene is not sufficient to produce normal developmental function and a "wild-type" phenotype.[27] In contrast, dominant negative effects are due to a mutant form of the gene product that interferes with the normal form and inhibits its function.[27] Both these phenomena are associated with similar developmental mapping functions that are concave upwards or sigmoidal (Figs. 1, 3; provided the wild-type developmental value and phenotype are scored as high values) and have similar consequences

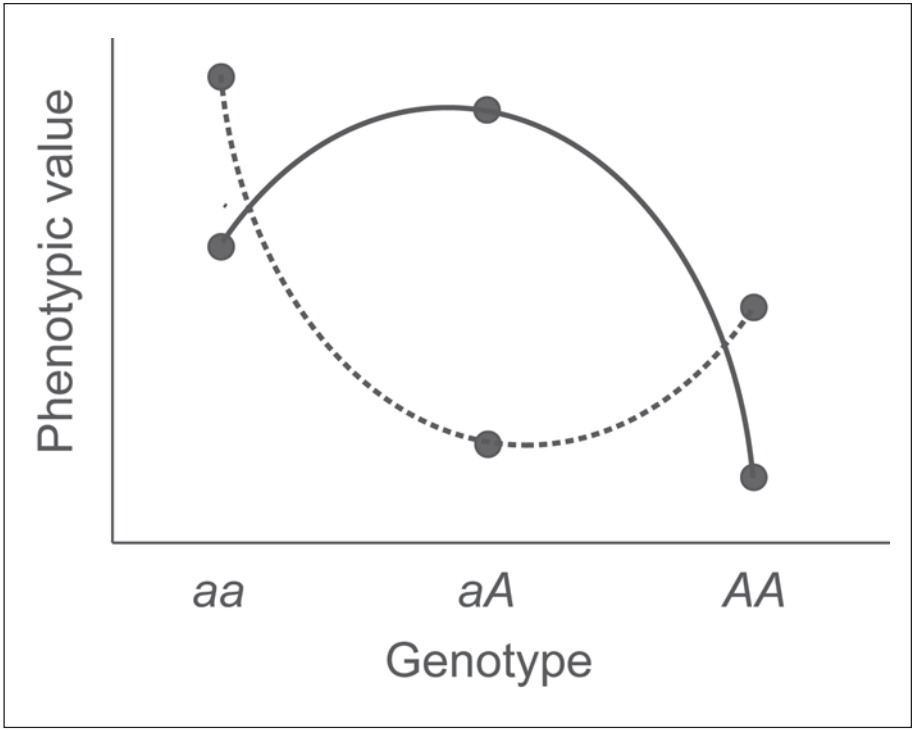

Figure 2. Overdominance and underdominance. For overdominance (solid line), the phenotypic value of the heterozygote exceeds the values of both homozygotes. For underdominance (dashed line), the phenotypic value is smaller than for either homozygote.

for developmental instability, and therefore need not be separated in the context of this chapter.

If more than one gene or developmental parameter is considered at the same time, the developmental mapping function becomes a surface over the space of developmental parameters. With two developmental parameters, this is a surface over the plane representing the parameters, where the elevation of the surface represents the phenotypic value. This kind of representation can easily be compared with a landscape, and various metaphors and techniques borrowed from cartography can be used to describe and visualize it. A simple way to recognize epistasis on such surfaces is to take slices through the surface parallel to the axis for one of the parameters, but set apart from each other along the axis of another parameter. The result is a comparison of developmental mapping functions for the first parameter at several distinct values for the second parameter. If these curves differ in their slope or curvature, and are not just transposed up or down, then there is an epistatic interaction between the two loci.[12,25]

Epistasis, the interactions among different loci where the genotype at one locus has an influence on the phenotypic effects of alleles at other loci,[30] is another result of the nonlinear nature of developmental mapping functions. Developmental systems are highly interactive, from the molecular and cellular level to the signals that achieve coordination of developmental processes throughout the organism, and the gene products of one locus can therefore directly affect the expression of other genes.[31,32] But even if there is no direct molecular interaction between the gene products of two loci, they still can affect each other's effects on the phenotype

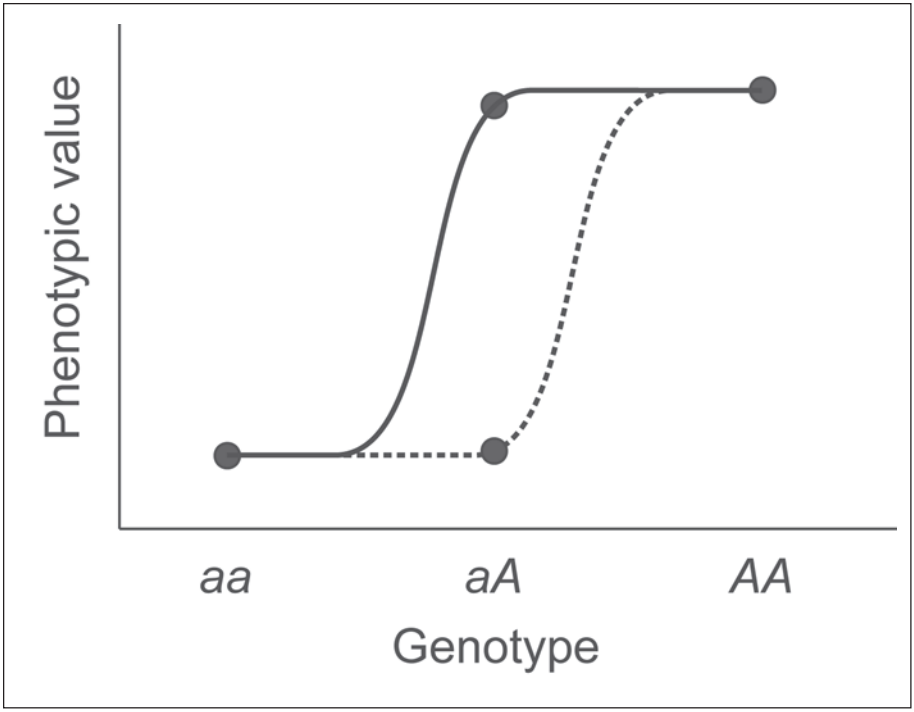

Figure 3. Sigmoidal developmental mapping functions. For these kinds of functions, the position of the genotypes along the horizontal axis is a critical determinant of the genetic behavior. The solid line shows a mapping function leading to dominance the A allele, whereas the dashed line, which has an inflection point at a higher value of the developmental variable, shows dominance of the a allele, and may be interpreted as a case of haploinsufficiency.[27]

by changing the shapes of the respective developmental mapping functions, to produce epistasis as this term is understood in statistical genetics.[30] Such epistatic interactions among genes are almost ubiquitous, even in "textbook examples" of simple Mendelian genes,[33] and have substantial effects on genetic variation of phenotypic traits.[34-36] Nonlinear developmental models produce epistasis among the loci controlling different model parameters almost inevitably.[12,25] Normally, these interactions cannot be eliminated by any one transformation of the phenotypic variable, which distinguishes them as genuine effects from simple scaling artifacts.[28]

Nonadditive Genetic Effects and Developmental Stability

The effect of random noise in developmental processes is that the values of the developmental parameters and the phenotype are not completely determined by genetic and environmental factors, because there is variation around the expected parameter values for a particular genotype and environment. In the context of graphs like those in Figures 1–3, random noise causes deviations of the developmental parameters to the left or right from the position corresponding to a particular genotype. Accordingly, the developmental system produces a slightly modified morphological result in response to this change of the input.

The magnitude of the phenotypic response to a given small amount of random noise in the developmental system depends on the local slope of the developmental mapping function (Fig 4). Where the mapping function is steep, a developmental perturbation will have a relatively large morphological effect, but where the mapping function is level, the same change of developmental parameters will have little morphological effect. Because developmental noise normally can be assumed to have effects that are small in comparison to the genotypic and environmental effects, a first-order approximation using the local slopes should be reasonably accurate as an estimate of the sensitivity of the system to random noise. This sensitivity to noise is a natural measure of the developmental instability of the system: its tendency to respond to developmental perturbation.[1,12]

Developmental stability, the system's ability to withstand developmental perturbations, is a consequence of the developmental functions that are relatively flat. This developmental stability does not necessarily rely on any buffering mechanisms that would actively oppose or compensate for variation in the developmental system, but the flat mapping function may indicate that some developmental parameters may not be relevant for the phenotypic response of the system under the given circumstances. This possibility has implications, for instance, for theories of developmental instability that assume the existence of metabolic or fitness costs of developmental buffering.[20] Developmental stability resulting from the structure of develop-

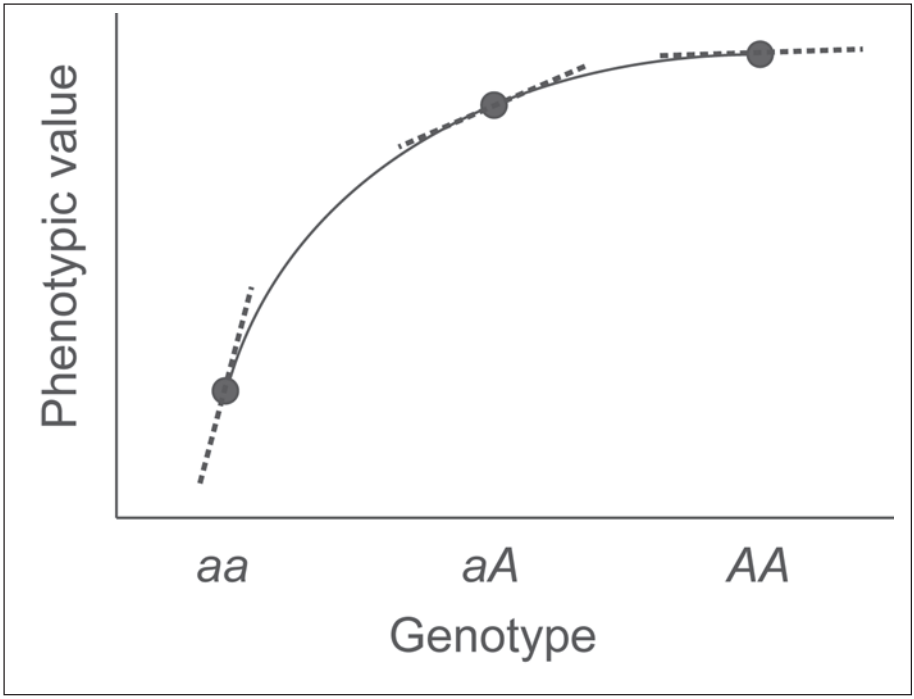

Figure 4. Slopes of the developmental mapping function at the locations corresponding to the three genotypes at a locus. These slopes are local indicators for the sensitivity of the phenotype to small perturbations of the developmental parameter, and are therefore a natural measure of developmental instability.[12] The three genotypes differ in their local slopes because the developmental mapping function is nonlinear (the A allele is partially dominant over the a allele). As a consequence, this locus has an effect on developmental instability.

mental systems may not be associated with any such costs, depending on the particular nature of the developmental system. This argument does not rule out the possibility that genuine buffering processes are involved in generating stability, for instance the well-known example of the Hsp90 chaperone protein.[8,9] Indeed, it is conceivable that the flat mapping functions themselves may be a result of buffering in some cases, but it should be emphasized that special buffering mechanisms are not the only basis for generate developmental stability.

For nonlinear developmental mapping functions, the local slope changes with the value of the developmental parameter, indicating that developmental stability varies with the value of the developmental parameter (Fig. 4). In particular, there will be genetic variation for developmental stability only if the slope changes sufficiently between the genotypes of a locus affecting the developmental parameter. This change of slope implies that the mapping function is nonlinear and therefore there also will inevitably be an appreciable degree of dominance for the trait value.[12] This link of dominance for the trait and genetic variation for developmental instability should be a fairly general phenomenon, occurring regardless of the specific mechanisms that generate the nonlinear developmental mapping function.

In particular, this argument suggests that any gene with a significant degree of dominance for the trait value will also have an effect on developmental instability. As dominance is widespread among the genes that have effects on phenotypic traits, it follows that numerous loci will also affect developmental instability. Therefore, there is no reason to assume a priori that the control of developmental stability and its measurable outcomes, such as fluctuating asymmetry, requires specific genes.[12]

The shape of the developmental mapping function has a substantial influence on the genetic effects of a locus on developmental stability. With partial dominance for the trait, the mapping function often is a "diminishing returns" curve, initially increasing steeply and eventually approaching an asymptote, its slope is steep initially and decreases gradually towards zero. Accordingly, the effects on developmental instability will have a substantial additive component with a smaller value for the allele that is partially dominant for the trait (i.e., the a value for developmental instability and d values for the trait will have opposite signs). In contrast, a mapping function that is sigmoidal with an inflection point near the heterozygous genotype will produce overdominance for developmental instability, because the slope at the position of the heterozygote is greater than that for either homozygote. And finally, loci with overdominance or underdominance of the trait will show underdominance for developmental instability, because the slope for the mapping function is minimal near the heterozygote (Fig. 2). Some caution is necessary, however, because the patterns of dominance for developmental instability depend on the precise shape of the mapping function of the respective trait. Moreover, in nonlinear developmental systems involving multiple loci, these patterns of dominance can be modulated by the epistatic effects of other loci, so that a precise prediction of the effects of individual loci is difficult.[12]

The results of empirical studies are consistent with these patterns, although the evidence is based on only a few study systems. A classical example is the case of increased asymmetry in Australian sheep blowflies carrying mutated genes conferring insecticide resistance, which can be reduced to normal asymmetry levels by a mutant allele of a single modifier locus.[37,38] Both the resistance and modifier genes are dominant in their effects on asymmetry.[38] Searches for quantitative trait loci (QTLs) for fluctuating asymmetry in mouse mandibles and skulls found relatively high degrees of dominance.[39,40] A study of two-locus epistasis among QTLs for fluctuating asymmetry of mandible size in the mouse revealed numerous epistatic interactions and a tendency for dominance effects to be involved in these effects (additive-by-additive epistasis was relatively rare).[41] All these studies consistently underscore the importance of nonlinear effects for the genetic architecture of developmental stability.

Evolution of Developmental Instability

Developmental stability may be under selection, even though there has been considerable debate on the relationship between fitness and instability measures such as fluctuating asymmetry,[20,42-44] and if there is genetic variation in a population, there will be he potential for an evolutionary response. Two simulation studies have investigated the evolutionary dynamics of developmental instability under selection and have produced results that emphasize the importance of nonadditive genetic variation in this context.[12,45]

Klingenberg and Nijhout[12] used a nonlinear model of a diffusion-threshold process controlled by six biallelic loci to define the trait values,[25] and small random deviations were added to the parameters of the model to simulate developmental noise in this system. Trait asymmetry was computed as the difference between two values generated with the same genetic contribution, but with different random values for the contribution of developmental noise. In this model, the genotype determined the developmental mapping function for each individual and thereby mediated the response to developmental noise, but there were no loci that controlled developmental stability directly and exclusively. For each generation, a population of 10,000 offspring was generated, from which 2,000 individuals were chosen to produce the following generation.[12] Whereas selection for the trait value led to relatively quick fixation of the favored alleles, selection of fluctuating asymmetry produced a substantially slower response. Indeed, the rate of change for fluctuating asymmetry was faster when selection was on the trait value, which was correlated genetically with asymmetry, than under direct selection for fluctuating asymmetry itself. There also was a difference between selection for increased and decreased values of the trait and, even more so, between selection for increased and decreased asymmetry. The response to downward selection was slower than upward selection. Under selection for decreased fluctuating asymmetry, the evolutionary change came to a halt after approximately 40 generations.

Nonadditive genetic effects played a substantial role in shaping the evolutionary dynamics in these simulated populations.[12] There was substantial epistasis among loci, through which the alleles of each locus tended to reinforce the effects of alleles at other loci, resulting in larger allelic effects in genetic backgrounds consisting mostly of alleles favoring increased trait values. Moreover, for most population compositions, there was a positive genetic correlation between the trait value and fluctuating asymmetry. Together, these properties of the genetic system led to the unequal responses to upward and downward selection. Under downward selection, epistatic interactions tended to erode the genetic variance by decreasing the allelic effects as alleles favoring small trait values became more abundant in the genetic background. In particular, downward selection on fluctuating asymmetry virtually eliminated all additive genetic variance in the population, so that there was no further response after approximately 35 generations.[12] The analyses suggest that the difference in selection response to upward and downward selection is largely due to the effects of epistasis. In addition, selection on fluctuating asymmetry is inherently less effective than selection on trait values because of the considerable random component of variation that makes an individual's observed asymmetry a poor predictor of the underlying degree of its underlying developmental stability.[46-49]

A different model was used by Fuller and Houle[45] in their simulation study of selection on fluctuating asymmetry. They assumed a genetic model for developmental instability that did not have any direct relation to the inheritance of the trait value.[45,50] Developmental instability was implemented as a variable with a lognormal distribution, which determined the variability of the trait around its expected value. Fluctuating asymmetry for each individual was computed as the difference between two values drawn from a normal distribution with this variance. Selection was simulated for one generation, and the results were compared for simulations with different heritabilities and amounts of variation for developmental instability. The

main result of the simulation was that selection was more effective for increased than for decreased asymmetry. In this model, this was due to the fact that that high asymmetry values are more informative, because they occur almost exclusively in individuals with a high degree of developmental instability, than low asymmetry values, which occur for all levels of developmental instability.[45] This result is consistent with the findings of the previous simulation of selection,[12] but adds an additional factor that contributes to the observed difference in the selection response to upward and downward selection. It is unclear, however, which of these factors is responsible for the failure to reduce fluctuating asymmetry in artificial selection experiments.[51]

Origins of Developmental Noise

Developmental systems are inherently "noisy" because they are not completely deterministic systems, but subject to random fluctuations.[52] These fluctuations originate from the molecular and cellular processes that constitute the system, and are therefore of substantial biological interest themselves. Rapid progress in the understanding of stochastic variation in gene expression, signal transduction, and other cellular processes has been made in prokaryotic and eukaryotic systems.[21,22,53-55] These random processes can contribute to variability of developmental processes and produce observable morphological variation.[1]

A number of studies have investigated stochastic variation in gene expression,[22,54,55] which may be taken as a proxy for developmental variation in general because of the fundamental importance of gene expression in developmental processes. Both transcription and translation stages have substantial influence on variability of gene expression. Transcriptional activity is related to the variability of gene product levels in variable ways in bacteria[22,54] and in a nonlinear manner with a maximum at intermediate activity in yeast,[55] presumably reflecting the differences in the transcriptional machinery between prokaryotes and eukaryotes. The variability of gene product levels has been found to be related to translational rate in a linear manner in both bacteria[54] and yeast,[55] suggesting that high efficiency of translation can amplify variation passed on from the transcriptional level.

Transcriptional processes are inherently variable because they tend to occur in bursts of activity interspersed with inactive phases.[56-59] A number of studies suggests that transcriptional regulation is achieved primarily by changing the probability of the "on" versus "off" states of the gene, rather than by adjusting the rate of transcription once the gene is activated.[56,57,60] Intermediate levels of transcription are achieved by alteration between the activated and inactive states, and therefore inevitably generate some variation in the mRNA and protein levels over time.[61] This mechanism is consistent with the finding that the variability of gene product levels tends to be greatest at intermediate levels of transcriptional activity.[55]

Cook et al[61] simulated the dynamics of stochastic gene expression in a model considering transcription, translation, and the decay of mRNA and protein product. The level of gene product is maintained at a constant average level by alternating phases of active transcription from the gene, during which the product levels will increase, with inactive phases when product levels will decrease because of the decay of mRNA and protein.[61] The precise dynamics of these processes depend on a multitude of cellular processes, but they can be summarized by the effects of the probability of the active versus inactive transcriptional state and the durations of these bouts of activity relative to the half life of the gene products.[61] If switching is fast, there will be little time for changes in gene product concentrations before the next change of transcriptional state and the ensuing reversal in the direction of change. In contrast, if switching is slow, levels will increase appreciably during the active phases and drop during the inactive phases. Therefore slow transcriptional switching results in an increased variation of gene product levels.[61]

The same model also makes predictions about the effect of gene dosage on the variability of gene product levels. Increasing the copy number of a gene has a similar effect as faster switching rate, because the chance that the different copies are active or inactive in synchrony decreases with increasing copy number. As a consequence, the variability of product levels relative to the average will decrease with increasing gene copy number.[61] Gene duplication can therefore have a stabilizing influence on developmental processes, as long as the paralogous genes are functionally equivalent.[62] In contrast, the loss of one of the two copies of a gene in a diploid organism may be associated with a substantial increase of the random fluctuations of gene product levels, which in turn may have serious consequences for the organism.[61] Indeed, this model and some extended versions of it were used by Cook et al[61] to explain the etiology of some haploinsufficiency conditions.

An experimental test of this model has been undertaken in a study of cell morphology of cultured melanocytes with different genotypes for the NF1 tumor suppressor gene.[63] The cell features studied were the number, lengths, and arrangement of the dendrites, which are long protrusions of these cells. Cells from skin samples taken from patients carrying mutations that inactivated one copy of the *NF1* gene, and where NF1 protein levels were reduced to approximately 50%, were compared to cells with two functional copies of the gene. The cells carrying the mutation showed increased variation in the number of dendrites and in the angles between dendrites, but there was no significant difference for dendrite length. Thus for two of the three cell characteristics considered, the predictions of the model by Cook et al[61] appear to hold.

These explanations of the molecular origins of stochastic variation in cellular processes are compatible, on the whole, with the approach based on nonlinear developmental mapping functions. The two approaches aim to explain the same phenomena, but are complementary in that the molecular models focus on specific mechanisms that generate random variation, whereas the framework of mapping functions is more phenomenological and concentrates on the transmission and expression of this variation in complex developmental systems. In both contexts, nonadditive effects play a substantial role via the consequences of gene dosage and through the nonlinear nature of developmental mapping functions.

Perspective: The Challenges Ahead

The Challenge of Multidimensional Phenotypes

The discussion in this chapter so far has focused entirely on the situation where the phenotype of interest is a scalar quantity, such as a length measurement, a count of repeated structural elements, or a scalar index representing a more complex aspect of morphology. Many phenotypes, however, are inherently multidimensional and cannot be easily reduced to a scalar index without severe loss of information. An example of such an inherently multidimensional phenotype is the shape of organisms or their parts, which can be quantified as a configuration of morphological landmark points[64,65] or the outline of the structure of interest.[66] In either case, the analysis of shape relies on the methods of multivariate statistics to extract relevant features of shape variation. Methods for the analysis of fluctuating asymmetry of shape are available and have been used in a variety of contexts.[67,68]

The developmental mapping functions for multidimensional phenotypes are more difficult to visualize than for scalar phenotypes, because they are not simple functions or response surfaces. The mapping function for a single input variable can be depicted as a trajectory in the multidimensional space, that is, the path on which the phenotype moves in response to the input value. This trajectory may be curved in the multidimensional space depending on the degree of nonlinearity of the developmental system. For two parameters, the mapping function is a surface, and for three parameters, it is a volume. As the number of parameters increases, this

approach becomes more and more cumbersome because the mapping functions cannot be visualized any more. It is possible, however, to derive some predictions for the statistical behavior of the variation, which can be compared to the outcome of experimental treatments or comparisons of variation at different levels.[4,69,70]

Multivariate analyses of fluctuating asymmetry and variation among individuals extract patterns of variation that correspond to particular directions in phenotypic space that can provide information about the developmental system. In particular, the patterns of fluctuating asymmetry, even though they originate from random noise in developmental processes, can provide information about the developmental origins of integration among traits and other questions.[71] In the context of the present discussion on developmental mapping, a question of particular interest is the dimensionality of morphological variation. Because stochastic variation occurs in most developmental processes to some extent, the variation should be distributed over all dimensions of the parameter space, unlike genetic or environmental variation, where the available variation depends critically on the experimental design or study population used. If asymmetry variation is concentrated in only relatively few dimensions of the phenotypic space, as has been observed in several studies,[67,69,70,72] this indicates that the developmental system is channeling variation into a subset of the possible patterns. These patterns should correspond to those directions in morphological space for which the developmental mapping function is particularly steep. Therefore, it is possible to make some limited inferences about the developmental mapping function even though the relevant developmental parameters are not accessible for most empirical studies.

Directions for Future Research

This chapter has reviewed some of the theory and empirical evidence for the role of nonlinear developmental mapping, as a factor influencing the stability of developmental systems. The link between dominance of traits and genetic variation of developmental stability for those traits has been established theoretically, but has only begun to be studied empirically. A number of new approaches have been used in recent studies, which have produced an understanding of developmental noise arising within these systems and its transmission to observable phenotypic traits.

A major challenge will be to identify the mechanisms involved in developmental buffering. The example of the chaperone protein Hsp90, for which a clear role in stabilizing developmental processes has been established,[8-10] currently appears unique as a specific buffering mechanism. This mechanism is different from most of the ideas discussed in this chapter because Hsp90 is a buffering mechanism that reduces variability for many morphological traits, apparently without affecting the average trait values.[8] It is unclear, however, whether there are other similar mechanisms and whether mechanisms other than those involving chaperone molecules also have similar buffering functions. Developmental buffering phenomena are not always attributable to specific genes, but they may also result from the organization of gene regulatory networks, whose multiple feedback interactions may provide an inherent degree of stability and facilitate the evolution of developmental robustness.[11,73,74] Again, the nonlinearity of the relationships in these networks is a crucial factor determining the behavior of these systems.

Another important task will be to link this mechanistic information on developmental stability to the phenotypes affected. A significant first step has been made by linkage analysis for fluctuating asymmetry of various kinds of phenotypes.[39-41,75] The reasoning that nonlinear developmental mapping is a primary determinant of developmental instability predicts that the same genes should affect traits and fluctuating asymmetry, but the available empirical data do not allow to assess this association conclusively.[12,76] A full understanding of the link be-

tween the mechanisms conferring developmental stability and its phenotypic expression will require much additional, innovative research.

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