A developmental perspective on developmental instability: theory, models and mechanisms

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Abstract

Although the terminology used in studies of developmental instability refers explicitly to development, this field of research has largely existed in isolation from mainstream developmental biology. Here I review the theory and models concerning fluctuating asymmetry (FA) with respect to the developmental mechanisms they assume. Some of the published models deliberately do not refer to developmental mechanisms at all, but are purely phenomenological models designed to examine statistical questions relating to FA. Other models are based on the dynamics of reaction–diffusion processes, and interpret the resulting oscillations in reactants as an analogue to the source of FA, and another model includes simplified model of both a developmental process and its genetic control. These studies emphasize the importance of feedback mechanisms and of nonlinear dynamics of developmental processes, and illustrate that fairly simple models are sufficient to generate patterns similar to those observed in empirical studies. In recent years, a number of models for stochastic switching of gene expression have been developed, which provide a possible explanation for developmental noise. Moreover, mechanisms such as gene duplication and stabilization of proteins by molecular chaperones possibly provide developmental stability, but many more are probably involved. These molecular models, which hold many possibilities for experimental studies of the mechanisms involved, point toward a promising new approach to understanding developmental instability that can reunite this field with developmental biology.

Introduction

The study of fluctuating asymmetry (FA) has become a burgeoning field in evolutionary biology (reviewed, e.g., by Palmer and Strobeck 1986; Palmer 1996b; Møller and Swaddle 1997). Most of these studies use FA as a measure of “developmental instability”, “developmental stability”, or “developmental noise”, but usually are less than explicit about the specific meaning of these concepts. Although these three key terms all contain the modifier “developmental”, however, it is unclear how the notions of instability, stability, or noise in theories of FA relate to the mechanisms that are the central issue of developmental biology. Indeed, it is unsettling to

realize how little is known on the actual processes that generate FA. How can we rely on FA if we don't know just how it works?

Empirical studies of FA for the most part are phenomenological rather than mechanistic. Even those studies that explicitly address the development of asymmetry in particular traits are mostly descriptive, or infer from patterns of morphological variation to the processes that generated them. A number of these are longitudinal studies following the asymmetry of individual organisms through a number of growth stages (Chippindale and Palmer 1993; Møller 1996; Collin 1997; Swaddle and Witter 1997; Aparicio 1998; Tomkins 1999), whereas others base inferences on within- or between-species relationships between FA and age or size (Hallgrímsson 1993; Teather 1996; Hallgrímsson 1998, 1999). Even a study that used experimental manipulation to generate asymmetry did not unambiguously identify the specific mechanisms involved (Klingenberg and Nijhout 1998).

FA studies, therefore, tend to be far removed from the mechanism- and gene-centered approach of mainstream developmental biology (for a standard text, see Gilbert 1997; for a more intuitive introduction based on metaphor, see Coen 1999). One unique exception is the finding that a homologue of the *Notch* gene of *Drosophila* acts as a modifier restoring normal levels of FA and fitness in Australian sheep blowflies carrying a mutation providing insecticide resistance (Batterham et al. 1996; Davies et al. 1996). Such information on the identity of genes provides a linkage to the wealth of information on the gene function in development, and therefore enhances our understanding of the mechanisms responsible for FA. Because the specific genes are unknown for other cases, however, it is better to start a general survey examining the developmental basis of FA from a theoretical perspective, and then to ask, in a second step, how information about underlying processes can be accessed from morphological data obtained by empirical studies.

A number of theoretical models of the origin of FA have been published, which are based on different concepts of developmental instability (Emlen et al. 1993; Graham et al. 1993a; Gavrilets and Hastings 1994; Leung and Forbes 1997; Klingenberg and Nijhout 1999; Van Dongen et al. 1999b; Houle 2000). These models were formulated for a variety of purposes, and therefore differ in many ways. Some are more phenomenological whereas others are more mechanistic, some are more realistic whereas others are more abstract.

Here, after a brief summary of the core ideas behind FA as a measure of developmental instability, I present an overview of these FA models from a primarily developmental perspective, which means that explicit consideration of mechanism is the principal criterion for judging the success of the models. None of them achieves high marks by this standard, but there are substantial differences among models in how much insight they provide into the origin of developmental instability.

Finally, I review the literature on stochasticity of cellular mechanisms that are involved in development, such as gene expression and macromolecular transport. These theoretical models and empirical studies address developmental processes at the level of individual cells and molecules (e.g., McAdams and Arkin 1999; Fiering et al. 2000), and have brought forward new and specific information on the origin of what the FA literature has referred to as developmental noise. On the one hand, therefore, these studies provide direct mechanistic explanations for a concept that has been rather vague and speculative. On the other hand, they make questions about FA amenable to the experimental approaches of developmental genetics and molecular biology. This reasoning based on mechanisms also points to new approaches to think about developmental stability, canalization, and homeostasis.

Developmental instability and asymmetry

The central argument: FA as a measure of developmental instability

The core idea of FA studies is that corresponding parts on the left and right sides of a bilaterally symmetric organism (or part of an organism, such as a symmetric leaf of a plant) are separate replicates of the same structure. The body sides of an individual share the same genome, and in a homogeneous environment, external effects on development are also the same on both sides. In an entirely deterministic system, corresponding parts on the left and right body sides would therefore develop the same morphology, as expected for the particular genotype in that environment. Accordingly, left and right body sides would be exact mirror images of one another.

Real developing organisms, however, are not deterministic systems. Even in a homogeneous environment, small random perturbations of cellular processes produce deviations of an organism's actual development from what would be expected given its genotype and environment. These perturbations — developmental noise — are a consequence of the stochastic nature of cellular processes (see below; e.g., McAdams and Arkin 1999). Because most of these processes act locally, a given perturbation usually will only have an effect on a small part on one body side (except for processes very early in embryonic development), and the effects of perturbations will accumulate in the developing organs on left and right sides separately. Unless there are compensatory mechanisms, the development of left and right body sides will therefore deviate from each other. The left-right asymmetry of morphological structures is the visible expression of the developmental noise that has accrued throughout development.

Developmental noise exerts its effects as development progresses. Because development is a system of highly interactive processes (e.g., Gilbert 1997; Coen 1999), perturbations occurring at one time can have significant effects on later developmental events, and thus the effects of developmental noise in different stages may not be independent. Moreover, different parts can mutually affect each other's development through inductive interactions. Accordingly, perturbations need not simply accumulate with time or over space, because nonlinear features of development, such as regulatory feedback circuits or the multiplicative nature of tissue growth, can dampen or amplify their effects.

Therefore, the sensitivity to developmental noise is an important property of the developmental system. This sensitivity, the tendency to produce a morphological change in response to a small developmental perturbation, can be interpreted as the organism's developmental instability (e.g., Klingenberg and Nijhout 1999). Its counterpart, the system's capacity to absorb developmental perturbations without eliciting a morphological response, corresponds to developmental stability. Developmental stability is a property of developmental systems that characterizes their buffering capacity or robustness, and it is thus closely related to the concept of canalization (see Nijhout, this volume; Waddington 1942; Wilkins 1997; Eshel and Matessi 1998; Gibson and Wagner 2000). Developmental stability and instability have traditionally been distinguished in the literature on FA, but they can be considered as the two sides of the same coin because they are opposite aspects of the way in which a developmental system responds to perturbations.

FA expressed in a morphological structure, in this view, is the result of developmental noise producing effects in the context of that particular system's developmental instability. Developmental noise *causes* differences between body sides, but whether and how these differences become manifest as morphological asymmetry is *mediated* by the organism's developmental instability. Extrinsic factors such as stress therefore can influence FA both through changes in the amount of noise and through changes of developmental instability.

Directional asymmetry as a measure of developmental instability

Left and right sides of most animals do not only differ by the small random deviations of FA. For instance, the internal organs of humans and other vertebrates show marked and constant differences between left and right body sides: they display directional asymmetry (DA). DA is by no means limited to vertebrates, but has been found for at least some organs in all major groups of bilaterian animals that have been investigated in this respect (e.g., Palmer 1996a; Wood 1997; Klingenberg et al. 1998). DA implies that the two body sides are systematically different in some respects. Especially in vertebrates, a considerable number of genes have been shown to be expressed only on one body side during specific periods in early embryogenesis (for recent reviews, see Tamura et al. 1999; Capdevila et al. 2000). Many of these asymmetrically expressed genes are signaling factors and regulatory genes, which not only control the morphogenetic events that produce the dramatic left-right asymmetries of internal organs, but also play important roles in the development of structures that superficially appear to be symmetric (e.g., the *sonic hedgehog* gene is important for patterning in the limbs). Because these genes and signaling pathways have the capacity to respond to differences in positional signals between body sides, it is conceivable that they may also be responsible for the more subtle directional asymmetry as it has been reported for structures such as mouse jaws (Leamy et al. 1997) or fly wings (Klingenberg et al. 1998). This hypothesis about the origin of the widespread directional asymmetries remains to be tested experimentally. It is clear however, that morphological structures on the left and right sides are not replicates grown under precisely identical conditions, as suggested in the previous section: there may often be a subtle difference in the positional signals expressed on the two body sides.

While DA and the difference in positional signals between body sides pose a challenge to the central argument underlying FA studies in principle, because corresponding structures on the left and right sides do not develop under exactly identical conditions, these issues are unlikely to cause serious difficulty in practice. The direct effects of DA can easily be accommodated: FA is taken to be the deviation of individuals' asymmetry from the average asymmetry (i.e., from DA expected for the genotype and growth conditions), rather than the deviation from "perfect" symmetry. In practice, subtracting the average asymmetry has long been inherent in the two-factor ANOVA customary in FA studies (Leamy 1984; Palmer and Strobeck 1986; Palmer 1994; Klingenberg and McIntyre 1998). If DA is correlated with the left-right average of the trait under consideration, other corrections are available (Graham et al. 1998).

I should caution, however, that adjusting of FA values by correcting for the average asymmetry is not a complete solution to the problem posed by DA, but perhaps is best thought of as something like a first-order approximation. The procedure makes the implicit assumption that the left and right sides are equally susceptible to developmental noise, that is, that the left and right sides have the same developmental instability. This is a reasonable assumption if DA is small: the developmental processes on the left and right sides are nearly identical, and accordingly, the system will respond to perturbations on either side in nearly the same way. If

there are conspicuous morphological asymmetries, it is possible that the asymmetries in development are so large that the two body sides cannot serve as intrinsic "controls" for comparison, making asymmetry unsuitable as a measure of developmental instability. In most FA studies, however, DA is subtle and the developmental differences between body sides are likely to be sufficiently small that FA correction by subtracting average asymmetry provides a good measure of the phenotypic effects of developmental noise and instability.

Some authors have suggested that it is possible to use DA itself as a measure of developmental instability (e.g., Graham et al. 1993a; Møller and Swaddle 1997, p. 17 ff.; Smith et al. 1997). Because DA is the mean asymmetry in a sample, it does not represent the morphological outcome of random perturbations due to developmental noise; to the contrary, it averages out that random variation and stands only for the systematic differences between sides. It is therefore fundamentally different from FA, and not and expression of developmental noise or developmental instability. If change in an environmental factor, such as a stress treatment, results in differences of DA, the treatments have unequal effects on the left and right averages, as the developmental response to the environmental factor depends on the positional signals that regulate the differences between left and right sides. Therefore, variation in DA with an environmental factor is a special example of a reaction norm, one that affects the two body sides differentially, but does not relate to developmental instability.

Models of fluctuating asymmetry

A survey of models

The models of FA published to date were set up for different purposes. The first group consists of mainly descriptive models that deals with primarily statistical questions, but do not explicitly consider the developmental mechanisms producing FA (e.g., Leung and Forbes 1997; Gangestad and Thornhill 1999; Houle 2000). These models examine, for example, how well developmental instability can be estimated from observing FA variation, given a specific statistical distribution, but considerations about mechanism are not the central concern. A group of similar models, based on models that are primarily statistical rather than biological, examine the change in FA during growth (Hallgrímsson 1998, 1999) or the genetic basis of FA (Gavrilets and Hastings 1994). In contrast, the remaining models address explicitly how FA is produced in the organism, as they explore questions such as the importance of feedback processes (Graham et al. 1993a; Van Dongen et al. 1999b) or whether specific "FA genes" are necessary to produce heritable variation (Klingenberg and Nijhout 1999). Because both groups of models are based on various assumptions about development, it is justified to evaluate these models by the way they incorporate information about developmental processes.

Statistical models of developmental instability

The simplest type of FA model gives a statistical description of FA in relation to some factor of interest, but is strictly phenomenological in that it does not make explicit reference to the mechanisms or processes responsible. A typical example of this approach is the model proposed by Leung and Forbes (1997). These authors are deliberately non-committal in their characterization of how FA is related to developmental noise (DN) and developmental stability

(DS), and offer two alternative mathematical formulations (p. 398). The first formula calculates FA by division, FA = DN / (1 + DS). The second version is a simple subtraction, |FA| = |DN| – DS, with FA set to zero whenever DS > DN. Considering the dimensions of the different variables shows just how much the two versions differ: in both versions FA and developmental noise are in the same dimension (for most FA studies, length), but in the division formula, developmental noise is dimensionless whereas in the subtraction formula it is also in the same dimension as FA. This discrepancy in dimensions indicates that developmental noise is of a fundamentally different nature in the two alternative formulas, and thus plays a different biological role. After brief discussion, Leung and Forbes drop the subtraction formula because it can generate perfect symmetry (p. 398), and concentrate on the division formula for the remainder of their paper.

In a further round of definitions, Leung and Forbes (1997, p. 398 f.) introduce factors that affect developmental stability. First, the value for developmental stability is specified as a linear function of individual quality (the authors seem to use quality and fitness interchangeably, p. 399). To define quality of individuals under stress (Q´) as a function of quality without stress (Q) and of stress level (S), Leung and Forbes again consider the alternative formulas by subtraction, Q´ = Q – S, and by division, Q´ = Q / (1 + S), as above. They favor the one by division because they "felt that an asymptotic relation is biologically more realistic" (p. 399). Stress, in turn, is defined to include any "factors which negatively impact quality or fitness" (Leung and Forbes 1997, Table 1).

The simulations show that even under the favorable conditions of deterministic relationships between stress, quality, and developmental stability, FA is of limited value as an indicator of quality or stress. It does provide information for individuals with unusually high asymmetry, which are likely to be of poor quality, but the more symmetric individuals can just as well be of low as of high quality.

A group of similar statistical models examines how reliably developmental instability can be estimated from FA (Whitlock 1996; Van Dongen 1998; Whitlock 1998; Gangestad and Thornhill 1999; Houle 2000; Leung et al. 2000). All these models use properties of the normal distribution like the association between mean and variance of the half-normal distribution (e.g., Whitlock 1996) or that mixtures of normal distributions with different variance produce leptokurtic distributions (Gangestad and Thornhill 1999); therefore, they rely strongly on the assumption that the signed left-right asymmetries for individuals of identical quality follow a normal distribution. These models agree in that they all find that FA is an inherently imprecise indicator of developmental instability, even if all model assumptions are met. This weakness may be alleviated to some extent by using FA of multiple, developmentally independent traits (Leung et al. 2000).

In sum, these models are built from assumptions about the statistical properties of FA and its relations to other factors such as stress and fitness, but do not consider the developmental processes that generate these relations. In their concluding remarks, Leung and Forbes (1997, p. 399) "emphasize that the processes which generate asymmetry values in our model reflect how FA is supposed to be generated, and that conclusions from this model should help refine emerging FA theory." To the extent it is justified, this statement highlights the degree to which developmental mechanisms traditionally have been ignored in the literature on FA.

Phenomenological models of the biological basis of FA

A second group of models addresses questions about the biological basis of developmental instability, but the models are similar to the preceding ones in that they are primarily statistical in nature. Gavrilets and Hastings (1994) offer a statistical model in which developmental instability is assumed to be a linear function of the genotype and an external "microenvironment" of every individual, which can correspond to stress or the individual's quality. The use of a linear function for genetic effects is equivalent to the assumption that they are entirely additive. This model produced a negative association between the variance induced by the "microenvironment" and heterozygosity, matching existing theories on FA and the findings of some empirical studies of the role of heterozygosity (Mitton 1993; Møller and Swaddle 1997, p. 122 ff.).

Similarly, Hallgrímsson (1998) presented models of the change in FA through growth, in which FA accumulates in an additive or multiplicative manner during the period of growth (see also Hallgrímsson 1999). FA is modeled as a normally distributed random variable whose standard deviation can scale with growth velocity or increase with age. Depending on particular variant of the model, the amount of FA, after correction for size, can increase or decrease. Hallgrímsson then compared these patterns of the time course of FA to empirical data sets on intra- and interspecific associations of FA with age and maturation period in mammalian bone. The conclusion of these studies was that FA tends to increase with age or maturation period, suggesting that bone remodeling either under the influence of asymmetric use or the accumulation of random perturbations are the principal sources of FA (Hallgrímsson 1998, 1999).

Reaction-diffusion model

An entirely different approach is to start from the theory and models of specific processes assumed to be similar to those actually involved in the development of FA. There is a considerable body of mathematical theory on pattern formation (reviewed, e.g., by Meinhardt 1982), which has been supplemented in recent years by more explicit models of morphogen action (Neumann and Cohen 1997; Kerszberg 1999) and of the control of gene expression (Bodnar 1997; Thomas 1998; Smolen et al. 2000; von Dassow et al. 2000).

In the context of FA, studies of this kind have used models of the nonlinear dynamics of reaction–diffusion processes to simulate the origin of FA (Emlen et al. 1993; Graham et al. 1993a; Van Dongen et al. 1999b). These studies rely heavily on the theories of chaotic dynamics in nonlinear systems and fractal geometry, and emphasize the sensitivity of biological systems to perturbations. Emlen et al. (1993, p. 85) explain that "the complexity of the multi-step, interlocking, multiple-chain enzyme pathways involved in molecular synthesis lead almost certainly to chaotic oscillations in growth". Moreover, "many, if not all, physiological processes in living organisms will be complex-cyclic" (Emlen et al. 1993, p. 85). Therefore, according to this view, small perturbations can evoke a large response, making the overall behavior of the system highly dependent on initial conditions and difficult to predict over the long term.

Within this general framework, Graham et al. (1993a) present a model of a reaction–diffusion system to investigate the role of feedback in the origin of the different kinds of morphological asymmetry. The model considers the concentrations of two morphogens: an activator and an inhibitor. The concentration of the activator substance is also the variable that directly serves as the output of the model for which FA is assessed (i.e., there is no

transformation into a morphological trait, or such a transformation is assumed to be linear). The activator and inhibitor exert their effects both on themselves as well as the other morphogen. The activator's effects on itself and on the inhibitor, and the inhibitor's effect on itself are modeled as linear functions of concentration, whereas the inhibitor's effect on the activator follows Michaelis-Menten kinetics. The model computes morphogen concentrations separately for the left and right sides, linked only by the inhibitor's ability to diffuse from side to side. Depending on the choice of parameter values, this model produces a variety of dynamic outcomes, including stable cycles and chaotic oscillations. Graham et al. (1993a) included developmental noise in their model by directly adding small random deviations to the concentrations of both morphogens at each step in the simulations. These simulations, depending on parameter values, produced unimodal or bimodal asymmetry distributions centered at zero, which correspond to the patterns expected for FA or antisymmetry (e.g., Palmer and Strobeck 1986). The simulations also confirm earlier theoretical results that feedback between left and right body sides is necessary to produce a bimodal asymmetry distribution, that is, antisymmetry (Van Valen 1962; Palmer and Strobeck 1992). Asymmetry distributions with a mean value other than zero, indicating DA, can only be produced by setting a parameter to different values on the left and right sides.

Van Dongen et al. (1999b) have extended this model to include two traits on each side, which can be correlated with each other. Therefore, the model has additional feedback interactions between traits on each side in addition to those between sides. Accordingly, the asymmetries resulting from the model may be correlated depending on the specific parameter values chosen.

These models show that the patterns of asymmetry observed in morphological traits can be generated by a fairly simple set of processes. Moreover, the models show that feedback between sides is necessary to generate a bimodal asymmetry distribution indicative of antisymmetry (Graham et al. 1993a) and that feedback between traits is required for correlations between signed asymmetries (Van Dongen et al. 1999b). Therefore, these studies identify necessary conditions for those patterns observed in empirical data.

It is unclear, however, to what extent these models reflect how actual developmental systems produce morphological asymmetries. First, the model treats the morphogen concentration as the outcome, and omits the mapping function to a morphological trait, which would require one more, probably nonlinear transformation. Second, and possibly more serious, is the notion of development as a labile system, in which small perturbations are sufficient to initiate chaotic oscillations. Oscillations resulting from feedback interactions do occur in specific contexts such as heart beat (Graham et al. 1993a), biological clocks (Shearman et al. 2000), or artificial oscillatory systems engineered from mutually repressing transcriptional regulators (Elowitz and Leibler 2000). However, they are largely unknown from the processes directly involved in the development of morphological traits. Indeed, studies in recent years have uncovered that the regulatory circuitry controlling gene expression, cell division, and cell growth tends to be quite robust against perturbations. Far from being sensitive to initial conditions, these systems are often remarkable for their ability to compensate even for major disturbances resulting, for instance, from genetic changes or experimental interventions (e.g., Barkai and Leibler 1997; Gerhart and Kirschner 1997, chapter 9; Little et al. 1999; von Dassow et al. 2000; Wagner 2000). Other nonlinear systems, such as developmental "toggle" switches, show stable behavior that is invariant under a wide range of conditions, and respond in a sensitive manner only near a sharp threshold separating the alternative domains of stable behavior (Gardner et al.

2000). The key component in all these systems is regulatory feedback, as emphasized by Graham et al. (1993a). Such feedback, however, need not lead to fluctuations in the behavior of the system, but can be inherently stabilizing itself (e.g., Becskei and Serrano 2000; von Dassow et al. 2000).

In conclusion, the models of Graham et al. (1993a) and Van Dongen et al. (1999b) identify conditions necessary for some of the patterns observed in empirical FA studies. They highlight the critical importance of feedback mechanisms, but they do not provide a realistic characterization of the actual developmental processes. However, in combination with more mechanistic approaches to modeling biological systems (e.g., Smolen et al. 2000), these models point toward a promising approach for further investigation.

Diffusion-threshold model including genetic control

Some investigators have hypothesized that FA and the left–right mean of a trait are controlled by different loci (e.g., Møller and Pomiankowski 1993b, a), and the genetic basis of FA generally has been controversial (e.g., Markow and Clarke 1997; Møller and Thornhill 1997). Therefore, it is of interest to examine whether modeling the genetics of FA from a developmental perspective can shed new light on this issue.

The model of the genetic basis for FA by Klingenberg and Nijhout (1999) extends work by Nijhout and Paulsen (1997), who present a simplified model of a developmental process consisting of morphogen diffusion and a threshold response. A point source releases morphogen, which diffuses into the surrounding tissue. In addition, there is a constant background of morphogen release throughout the tissue, and the morphogen decays in proportion to its local concentration. After a set time, the local morphogen concentrations are compared to a threshold value, and a morphological trait is defined by the distance from the point source within which the morphogen concentration exceeds the threshold. Nijhout and Paulsen combine this developmental model with a quantitative genetic approach by assuming that each of the six model parameters is controlled by a single locus with two alleles in a strictly additive manner (i.e., the genotypic value of the heterozygote is midway between the two homozygotes). Any non-additive genetic effects at the level of the morphological trait is therefore an outcome of the developmental system. This genetic system shows interesting behavior in simulations of directional selection within a population (Nijhout and Paulsen 1997).

The extension of this model for FA adds a small component of random variation to each developmental parameter to simulate developmental noise (Klingenberg and Nijhout 1999). This random noise component is normally distributed around a mean of zero, and it is independent of the genotype. For each individual, or genotype, left and right sides are simulated by two replicate runs with separate random values for the noise component, and FA is computed from the difference between them.

In a first step, quantitative genetic parameters were estimated from 1000 individuals of each of the 729 genotypes possible with the six biallelic loci (Klingenberg and Nijhout 1999, fig. 1, tables 2, 3). These analyses show dominance and marked epistasis for the phenotypic trait. The effects of epistasis are such that those alleles that increase the left–right average of the trait (“Large” alleles) also tend to enhance the additive and dominance effects of other loci. Moreover, different genotypes respond to developmental noise with different sensitivities, producing genetic variation of FA. For five of the six loci in the model, the Large alleles increase FA. FA displays marked dominance, with mostly negative dominance coefficients, indicating that the heterozygote tends to be less asymmetric than the average of the two homozygotes at a

given locus. And finally, there is marked epistasis for FA as well. In sum, dominance and epistasis are pervasive (cf. Leamy, this volume). They result from the nonlinearity of the diffusion–threshold model, which, even though the developmental parameters are controlled in a strictly additive fashion, introduces interdependence between the phenotypic effects of different alleles and different loci. These nonlinearities also produce interactions between genetic and nongenetic causes of variation even without specific "genes for FA": the genetic component of FA results from the genetically modulated expression of variation that is itself entirely nongenetic.

Further analyses simulate genetic variation in a population to which selection is applied, in both directions, on either the left–right average of the trait or on FA (Klingenberg and Nijhout 1999, Fig. 2–5). Selection on the left–right averages of the trait is more effective at changing the mean and allele frequencies than selection on FA. This result is not surprising, because FA has a substantial non-genetic component of variation in this model that is not present for the left–right trait average. Moreover, selection for higher left–right average and higher FA levels is more effective than selection for lower values because of the strong epistasis. During upward selection, the increasing frequencies of Large alleles enhance the effects of other loci, ensuring the persistence of genetic variance of the traits even as allelic variation is removed by selection. During downward selection, in contrast, Large alleles are removed from the population, diminishing the phenotypic effects of remaining variation at other loci, and thus reducing the effectiveness of selection. The correlations between heterozygosity (the number of heterozygous loci per individual) and FA are weak in most simulation runs. Finally, under all selective regimes, the phenotypic and additive genetic correlations between FA and left–right average of the trait are positive during most of the simulation.

Despite the oversimplified model, the simulations of FA in populations under selection have produced patterns found repeatedly in empirical studies of natural populations, such as the correlations between heterozygosity and FA or between trait size and FA. It is important to note that the simulation results do not imply that these patterns are produced by these processes in nature, because different mechanisms may produce similar results. The results indicate that the model's assumptions about the developmental system and its genetic control are *sufficient* to generate the observed patterns.

Implications of the models for asymmetry studies

It is possible, however, that a more general class of developmental models can also produce the results of the studies summarized above. In this section, I discuss this possibility and explore the broader biological context of these models. What emerges is a number of important lessons for empirical FA studies.

Nonlinear developmental mapping and FA

A useful way to think about the role of developmental processes in the context of FA is that they act as a mathematical function that maps a series of inputs such as the genotype, environment, and random noise to the morphological outcome. I call this function "developmental mapping", showing its affinity to the genotype–phenotype map of Wagner and Altenberg (1996), but I explicitly include non-genetic inputs in addition to the genotype.

A simple example is shown in Figure 1a, where the developmental mapping is a curve that relates the phenotype to a single input, such as the physiological activity of a gene (e.g., its expression rate or the activity of its protein product). This simplified example has a single input, which can be graphed along a single axis in a diagram, and the nonlinearity of curves such as that in Figure 1a has long been used in explanations of dominance for single loci (Kacser and Burns 1981). In real developmental systems, however, there are many simultaneous inputs, and in such a multidimensional system, nonlinearity of gene expression systems causes interactions between the activities of different components, as is apparent in epistasis between different loci (e.g., Omholt et al. 2000). The developmental mapping function results from the combined dynamics of all the components in the developmental system.

Inputs of variation from genetic and other sources into a developmental system exert their effects in combination, and it may often be impossible to separate contributions of the various inputs to the effect on "downstream" processes. For instance, whether the expression of a signaling protein is variable because of differences in the regulatory elements of the gene or because of an environmental influence, the outcome will be a change in the amount of the protein produced, and in the strength of signaling to downstream processes. As a result, it will usually be exceedingly difficult to disentangle which part of the variation in a morphological trait is genetic, environmental, or due to developmental noise (see also Sarkar 1998, pp. 181 ff.; Gifford 2000). In the present context, therefore, I focus on how the developmental system acts as a conduit for variation, no matter what its origin. The developmental mapping characterizes this input–output relation.

Developmental mapping is linear in some FA models (e.g., Gavrilets and Hastings 1994; Gangestad and Thornhill 1999), but it is nonlinear in those models that explicitly take into account the underlying developmental processes (Graham et al. 1993a; Klingenberg and Nijhout 1999). Nonlinear developmental mapping raises some fundamental issues that are relevant to FA research. Even if perturbations themselves are random and occur independently from one another, the nonlinearity of the developmental system will cause statistical interactions of their phenotypic effects with genetic and environmental factors. Because of these interactions between developmental noise and genetic factors (and possibly other factors), it is not possible to disentangle genetic and nongenetic contributions to FA (for a more detailed discussion in a different context, see Sarkar 1998, chapter 4).

Normal distribution

As a consequence of nonlinear developmental mapping, the effects of developmental perturbations producing FA at the phenotypic scale are not "random, independent, and cumulative" (Palmer and Strobeck 1992, p. 59), as has been assumed to ensure normal distribution of asymmetry and left–right averages (e.g., Palmer and Strobeck 1992; Whitlock 1996; Houle 1997; Rowe et al. 1997; Van Dongen 1998; Gangestad and Thornhill 1999; Van Dongen et al. 1999a; Houle 2000). The observation that the normal distribution often fits the data well is of little help, because it can be difficult to decide whether empirical data fit between alternative distributions, even if the comparison is between a symmetric and a skewed distribution, such as the normal and log-normal distributions (e.g., Mosimann and James 1979). Nonlinear developmental mapping can produce distributions of trait values on left and right sides that are skewed or otherwise deviate from a normal distribution. Accordingly, the distribution of asymmetries will also not be normal, but show features such as leptokurtosis (Palmer and Strobeck 1992). Therefore, nonlinear developmental mapping violates a basic assumption of FA

studies that use properties of the normal distribution as a diagnostic tool for discovering heterogeneity of developmental instability within populations (see the cautionary remarks by Houle 2000, p. 728 f.). To the extent that nonlinear developmental mapping prevails (and the evidence suggests it is very widespread), the normal distribution is perhaps best viewed as a mathematically convenient approximation of the real phenomena that should be used with the utmost care.

Dominance and the FA–heterozygosity relation

Dominance is another consequence of nonlinear developmental mapping. In their model for the biochemical basis of dominance, Kacser and Burns (1981) studied the dynamics of a metabolic pathway composed of several enzymes, but other models where the developmental mapping is a diminishing-returns curve can serve the same purpose (Fig. 1a; Klingenberg and Nijhout 1999). Consider a gene with two alleles whose proteins products have different levels of activity in the cell: a wild-type allele with full activity, and a mutant allele with a reduced level of metabolic activity. The activity level in a heterozygote's cells is intermediate between those of the two homozygotes, because the heterozygote has 50% of each protein form. However, because the developmental mapping is a curve that is steep only at low activities and then quickly flattens toward an asymptote, the heterozygote has a phenotypic value that is much closer to the high value of the wild-type than to the mutant homozygote. Therefore, the trait will show nearly complete dominance of the wild-type over the mutant allele.

At high levels of activity, there is little change of the phenotype in response to a small change in gene activity, indicating that the system has a high degree of stability. In contrast, at low activity levels, small changes cause a much larger response, indicating that the system will respond to perturbations more strongly. In the presentation of the developmental mapping function as a curve of the relationship between gene activity and the phenotype (Fig. 1a), the slope is a direct measure of developmental stability (gray lines in Fig. 1a; Klingenberg and Nijhout 1999). Therefore, the developmental stability for every value of gene activity is provided by the derivative of the curve (Fig. 1b).

The argument for explaining dominance applies again, but this time for developmental instability as indicated by the derivative of the developmental mapping curve (Fig. 1b; Klingenberg and Nijhout 1999). Because the developmental mapping curve is fairly level in the region of the heterozygote and the homozygote for the dominant wild-type allele, and only is steep near the homozygote for the recessive mutant allele, the heterozygote and wild-type homozygote have similarly low values for the derivative. Therefore, the heterozygote and wild-type homozygote have a similar low degree of developmental instability, which is substantially less than that of the mutant homozygote. This means that the wild-type allele is not only dominant for its high phenotypic value, but also for its low value for the derivative. Notably, the level of developmental instability of the heterozygote is less than the *average* of the levels of the two homozygotes. This is a possible alternative to existing explanations for the association between heterozygosity and reduced FA (for discussion, see Clarke 1993; Mitton 1993; 1997, p. 98 ff.; Møller and Swaddle 1997, p. 119 ff.; Klingenberg and Nijhout 1999).

This reasoning relies exclusively on the curvature of developmental mapping, and therefore applies to a broad range of situations, far beyond any one specific model. For instance, the same pattern holds whether the dominant allele is associated with a higher or lower trait value than the recessive allele. If there is over- or underdominance for the trait, the developmental mapping is a ∩- or -shaped curve, respectively, and in both cases

developmental instability will display underdominance—there will be less FA for the heterozygote than for either of the homozygotes. In general, any nonlinear developmental mapping has the potential to generate genetic variation for developmental instability. The strength and specific form of genetic control depends on how exactly the genotypic values for homozygotes and heterozygote at a locus deviate from a linear arrangement. This means that genetic variation for developmental instability is closely tied to the precise form of dominance for the trait in question.

The reasoning outlined in this section is very general, and is likely to apply for many partial or complete loss-of-function mutations (e.g., "gene knockouts") as they are often used in developmental genetics. This argument does not hold in other cases, however, such as dominant mutations (e.g., Mayo and Bürger 1997), as in the case of the *Rop-1* pesticide resistance gene and the *Modifier* for asymmetry in the Australian sheep blowfly (McKenzie and Clarke 1988). It also remains to be investigated to what extent this reasoning applies to genetic variation in natural populations, which often consists of two or more wild-type alleles whose protein products have very similar rates of activity, and therefore may have different patterns of non-additive effects (cf. Leamy, this volume).

Epistasis and coadapted gene complexes

If, as part of a developmental system, the gene products of two or more loci interact, it is likely that both the left–right average values and developmental instability of the corresponding morphological traits will show epistasis between those loci (discussed extensively for FA under the heading of "coadapted gene complexes"; e.g., Clarke 1993). Because developmental systems are usually nonlinear, the loci can mutually influence each other's phenotypic effects in many ways. For two-way interactions, this developmental mapping is a function that can be visualized by a curved surface over two perpendicular axes representing the activity levels for the two genes; profiles intersecting the surface parallel to the axis for one locus will differ from each other depending on the activity level of the other locus. For multi-way interactions among loci, the developmental mapping can no longer be visualized, but must be treated mathematically.

Even for simplified developmental systems such as the diffusion–threshold model with six loci (Klingenberg and Nijhout 1999) or simple gene regulatory networks (Omholt et al. 2000), the multiple epistatic effects can be extremely difficult to disentangle. The only feasible option is therefore to use the methods of quantitative genetics and fit a simplified model of pairwise interactions to the data, as it has been done in QTL studies of left–right means of traits (Cheverud and Routman 1995; Routman and Cheverud 1997) as well as for FA (Leamy, this volume).

A particularly clear example of the genetic basis of such interactions is a modifier that interacts with an insecticide resistance gene in the Australian sheep blowfly, *Lucilia cuprina* (reviewed by Batterham et al. 1996; Clarke 1997). Flies carrying the *Rop-1* mutation, which confers resistance to the insecticide diazinon, have higher FA of bristle counts and lower fitness than susceptible flies, if reared without the insecticide. However, a *Modifier* mutation, which appeared in natural populations, returned bristle FA and fitness of *Rop-1* flies to the normal levels (McKenzie and Clarke 1988). This modifier is a homologue of the *Drosophila Notch* gene (Davies et al. 1996), which encodes the key receptor protein in a cell–cell signaling pathway controlling cell fate in many contexts (e.g., Artavanis-Tsakonas et al. 1999). Among many other functions, the Notch signaling pathway is important in bristle specification, and significant portions of genetic variation in bristle number in natural *Drosophila* populations have been

associated with two other genes interacting with *Notch* in this pathway (Long et al. 1998; Lyman and Mackay 1998). This case is unusual in terms of the magnitude of the effects of *Rop-1* and the *Notch* homologue in the sheep blowfly. It clearly confirms, however, that the same genetic interactions that are known to be involved in the "ordinary" development of a trait also can play a major role for FA. In other words, there need not be special genes or signaling mechanisms for FA.

Molecular mechanisms responsible for developmental instability

A key question that has attracted surprisingly little attention in studies of developmental instability and FA concerns the processes responsible for developmental noise or the mechanisms establishing developmental stability in the face of random variation. A number of authors have referred to thermal noise and other random variation as the origin of developmental noise (e.g., Palmer 1996b), but there have been no detailed studies of the mechanisms responsible, and how this variation in developmental processes is expressed in the phenotypic traits usually considered in FA studies. This issue has been addressed, however, by a rapidly growing body of literature in molecular biology and biophysics, which deals with stochastic processes involved in gene expression (e.g., reviews by Ko 1992; McAdams and Arkin 1999; Fiering et al. 2000; Smolen et al. 2000). Stabilizing mechanisms have been at least as enigmatic in the FA literature, but again, empirical and modeling studies at the molecular and cellular level have offered suggestions concerning possible mechanisms (e.g., Becskei and Serrano 2000). In the remainder of this chapter, I therefore review this literature, and discuss how models of molecular processes can shed new light on traditional questions in studies of developmental instability.

Origins of developmental "noise"

Developmental changes are based on processes at the cellular and molecular scale that are, albeit less obvious, no less dynamic than those at the morphological level. Cells and even nuclei provide a highly structured environment within which specific processes take place at particular locations and times. Technological advances have made it possible to study the dynamics of these processes in individual living cells and to visualize even single macromolecules (Femino et al. 1998; Rutter et al. 1998).

Simply the minute scale at which these reactions occur can make them very different from the biochemical reactions in a test tube of tissue homogenate that are more familiar to most biologists. Cells may contain only a few molecules of each kind. For genes that occur in a single copy per haploid genome, transcription must therefore use the two DNA molecules present in each diploid cell. Likewise, the messenger RNA transcribed from these genes is also present in a fairly modest number of copies, for instance, cultured cells contain RNA transcripts for the cytoskeletal protein β-actin in 500–1500 copies (Femino et al. 1998). Regulatory genes such as transcription factors, which are key players in all developmental processes, are present in much lower copy numbers. For instance, in polychaete embryos and larvae, the RNA transcripts of several *Hox* and the *Brachyury* regulatory genes are rarer by one to two orders of magnitude than those of the cytoskeletal β-tubulin (Peterson et al. 2000). Likewise, in sea urchin embryos, 30–100 transcripts per active cell have been estimated for some *Hox* genes, whereas others only

occur in 130 to 330 copies per entire embryo (Arenas-Mena et al. 1998). The number of molecules of regulatory proteins can be considerable, however, as illustrated by one study that estimated the number of regulatory molecules necessary for activating two genes in the order of 3×10^5 and 10^6 per nucleus (Shimizu and Gurdon 1999). Finally, processes such as intercellular signaling also can involve small to moderate number of molecules: two critical thresholds for the number of cell surface receptors that need to bind signaling molecules in order to cause switching of gene expression have been estimated at 100 and 300 molecules per cell (Dyson and Gurdon 1998). At least for the rarer among these types of molecules, the low copy numbers may cause random variation in the dynamics of processes in which they take part.

One source of random variation is delay in molecular interactions due to the transport between the cytoplasm, where protein synthesis takes place, and the nucleus, where regulatory proteins interact with DNA (Smolen et al. 1999). When a limited number of molecules is involved, transport by diffusion will produce delays that differ randomly among cells. For instance, it is a matter of chance how long it takes for a transcription factor of a specific kind to arrive at a free binding site in the regulatory region of a given gene, even though diffusion of protein molecules throughout the nucleus is remarkably fast overall (Phair and Misteli 2000).

This variability in the timing also relates to ideas about the stochastic nature of switching genes between "on" and "off" states. Studies of gene expression in individual cultured cells have produced increasing evidence that transcription of genes occurs in bursts at irregular intervals rather than at a constant rate (Ko 1992; Ross et al. 1994; Wijgerde et al. 1995; Fiering et al. 2000). Enhancers, regulatory elements usually located at some distance surrounding the coding region of a gene, increase the probability that a gene is activated, but not the rate at which it is transcribed once it is switched on (i.e., the rate at which RNA polymerase complexes are initiated at an active promoter site; Walters et al. 1995). The concentration of gene products in a cell therefore results from the dynamics of transcriptional switching, translation, and the decay of RNA transcripts and protein over time (McAdams and Arkin 1997; Cook et al. 1998).

I will use a simple model of gene switching (Cook et al. 1998) to explain how this stochastic variation of gene expression can generate developmental noise. Consider a system with a gene that can be either in an inactive "off" state or in an active "on" state, in which the gene is transcribed at a set rate (a constant amount per unit of time). The resulting gene product (for simplicity, I only consider a single product pool, rather than distinguishing RNA and protein, etc.) is decaying at a constant rate (a constant percentage per unit of time). In a diploid organism, there will be two copies (alleles) of the gene per cell, and I assume that both can switch on and off independently, but according to the same overall regime.

If both copies of the gene are switched from the "off" to the "on" state permanently, the product concentration increases until it reaches a constant value at the equilibrium point between production and decay. If the switch is not permanent, however, but expression from each allele is intermittent, then the product concentration will fluctuate around an average value, usually falling while both alleles are "off" and rising while both are "on" (with one allele on and the other off, the behavior of the system will depend on the concentration). Because persistence of the product leads to averaging over time, the rate of switching should be seen in relation to the product's half-life: stability of the product, as measured by its longer half-life, is a stabilizing factor in this system (the stability of RNA and protein products is also the primary reason why it is extremely difficult to study the expression dynamics of genes in natural systems). Imagine now that this system of unstable gene expression is changed so that switching is faster, but that the ratio of time spent in the "on" and "off" states is still the same. As a result, the average

product concentration will not change, but the fluctuations around this average will be smaller. Therefore, faster switching between “on” and “off” states contributes to the stability of the system.

If the activity of one copy of the gene is lost, for instance by mutation, the average concentration will decrease to about 50% of the normal level in a cell with two functional alleles (assuming there is no compensation for dosage). Moreover, the variation around the new average will increase too, because there is no second allele whose activity provides buffering. The extremes in product concentration, at the end of relatively long periods in the “on” or “off” state, therefore tend to deviate farther from the average concentration. Specifically, there will be a marked increase in the probability that the level of gene product will transiently fall far below half the normal average, say 10% of the normal level, to values that are hardly ever reached in the presence of two functional alleles. Cook et al. (1998) use this reasoning to account for haploinsufficiency (phenotypes associated with the inactivation of one allele in diploid organisms): if a visible phenotypic effect only appears if the product concentration falls below a particular low threshold value, then it will occur only in individuals missing at least one functional allele. Essentially, cells in heterozygous individuals will intermittently experience levels of the gene product as if they were homozygous for the loss-of-function allele (in the case of autoregulatory genes, this inactivation can become irreversible; Cook et al. 1998, fig. 2).

But why is it that genes are expressed at all in this unstable manner, switching between “on” or “off” states? Wouldn’t it be better for them to be either constantly inactive or constantly active, which would not cause problems in heterozygotes because of the variation associated with the unstable mode of gene expression? Cook et al. (1998) show one advantage of unstable gene expression with an extension of their model. The gene now responds to a an activating factor, that is, switching on the gene requires binding of an activator molecule (e.g., the product of a different, regulatory gene). With unstable expression in such a system, each time the gene is switched off, an activator molecule needs to bind in order to switch the gene on again. In every cell, there are therefore many switching events for its two copies of the gene, and each time the system is assessing the activator concentration. As a result, the level of the gene product can yield a more precisely defined response to the activator concentration than a similar system with stable gene expression (particularly with an imprecise mechanism for measuring activator concentration). This response is better defined in a system with a fast switching rate than in a system with slow switching. This clearly is of great importance for many developmental patterning processes, where the precise readout of concentrations in a gradient of some signaling molecule determines size and shape of the resulting morphological structures (Lawrence and Struhl 1996; Neumann and Cohen 1997; Gurdon et al. 1998). In this stochastic gene expression system, the loss of function of an allele substantially impairs the response to an activator (Cook et al. 1998, fig. 3). With only one functional allele, the system is less sensitive and the response increases not as steeply with increasing activator level, and therefore would result in less sharply defined spatial patterning.

Whereas Cook et al. (1998) discuss the results of their simulations in terms of haploinsufficiency, the same reasoning is also applicable to developmental instability and its genetic and developmental basis. Here I apply the same logic in the context of phenotypic variability in mutations that reduce gene function (partial or complete loss-of-function), which should help to understand how developmental noise from stochastic gene expression manifests itself in the phenotype.

A common observation in organisms bred for genetic experiments is that mutant phenotypes are not only morphologically different from the normal "wild-type", but also more variable. For instance, in *Drosophila melanogaster*, flies homozygous for the *abrupt*^{1} mutation (*ab*^{1}) have L5 wing veins that do not reach the rear wing margin, as in the wild type, but end at some distance from it (cf. Fig. 2a, b; e.g., Hu et al. 1995). There is considerable variability in the phenotype of *ab*^{1} flies, even in inbred laboratory stocks, ranging from strong expression to weak phenotypes indistinguishable from the wild-type. Moreover, even the two wings of a single fly can differ substantially in the expression of the mutant phenotype (Fig. 2c). This variation within individuals makes it very unlikely that variation in the environment or genetic background is responsible, and indicates that there is inherent instability in the expression of the mutant phenotype. The model of unstable gene expression provides a simple explanation for this finding. Flies homozygous for *ab*^{1} have functional Abrupt protein product that fulfils developmental functions for which it is essential (other mutations of the gene are recessive lethal; Hu et al. 1995), but presumably, the mutation reduces gene expression in the developing wing. The level of activity in mutant homozygotes appears to be reduced to just below the threshold level necessary for normal differentiation of the L5 wing vein, so that the distal part of this vein is missing in the typical mutant phenotype. However, the variability in gene expression is sufficient to surpass the threshold of activity for vein differentiation in a fraction of cases, and the resulting wings differentiate complete L5 veins, as in the wild-type.

This explanation for variable phenotypic expression may also apply to many other mutations, and it is not restricted to mutations with large effect. The model of Cook et al. (1998) should apply to a much broader class of reductions in gene expression than just the complete loss of activity of an allele (as in the considerations on haploinsufficiency). To the contrary, the model implies a general relation between decreasing gene expression and increasing variability.

Changes of gene expression due to many factors can all potentially affect variability in the level of a given gene's product, notably the presence of enhancer elements and the activity of regulatory proteins interacting with them (e.g., Walters et al. 1995; Arnone and Davidson 1997; Yuh et al. 1998). It follows that there is great scope for epistatic interactions among different loci (see Leamy, this volume; Omholt et al. 2000). Any gene that significantly affects the expression of one or more genes with phenotypic effects, and therefore has an effect on the average phenotype, has also the potential to affect developmental instability. In this respect, the conclusions drawn from the model of unstable gene expression (Cook et al. 1998) are similar to those from the model of a diffusion–threshold process (see above; Klingenberg and Nijhout 1999) and other models resulting in diminishing-return curves (see Fig. 1 and discussion above; Kacser and Burns 1981; discussion in Klingenberg and Nijhout 1999). All these models agree in that there appears to be no clear distinction between "trait genes" and "FA genes."

Buffering: mechanisms contributing to developmental stability

Developmental stability is the tendency of morphological traits to resist the effects of developmental noise, given an organism's genotype and particular environment. Canalization, on the other hand, is the ability to develop the same phenotype despite genetic variation or differences in the environment. Both developmental stability and canalization therefore refer to the developmental system's ability to buffer against perturbations; the difference between the two concepts is in the origin of the perturbations (see also Nijhout, this volume). Moreover, mechanisms that contribute to buffering of developmental systems should be effective regardless

of the origins of perturbations. A mechanism that makes a developmental system less sensitive to activity changes in one of its components should have a stabilizing influence, regardless of whether the change was originally caused by stochastic variation within the system, a genetic change in the regulation of that component, or a perturbation from the environment (see also Gibson and Wagner 2000). Therefore, it seems reasonable to suppose that the same processes contribute to both developmental stability and canalization, although this issue is contentious, with some authors going as far as claiming that they are independent (Debat et al. 2000). Here I briefly review several possible mechanisms for developmental buffering. To what extent these mechanisms are actually involved in producing developmental stability (or canalization) needs to be addressed experimentally.

There are several means by which developmental noise can be reduced or its expression dampened. One way to reduce stochastic variation in the expression of a gene is increasing the copy number of the gene. Cook et al. (1998) used their model of unstable gene expression to show that doubling the number of functional alleles from two to four substantially decreases the variation in the resulting product concentration. As there are more functional copies of the gene, the random variation in any one of them will have less influence on the total activity summed over all alleles present. This buffering effect does not require that the genes are exactly the same, but it is sufficient that they can substitute for each other's function to some degree, so that even partial redundancy of gene function (e.g., groups of paralogous genes) has a stabilizing influence on developmental processes (e.g., Wilkins 1997). Population genetic models suggest that this role, besides other processes, may contribute to the evolutionary conservation of redundant gene function and duplicated genes (Krakauer and Nowak 1999; Wagner 1999).

Besides an increase in gene copy number, the other factors discussed previously as influencing developmental noise, when applied in the opposite direction, can also contribute to developmental stability. Increases in the numbers of regulatory protein molecules should reduce the variation in the time to initiation of transcription (McAdams and Arkin 1999). This is part of an increase in the rate of gene switching, which leads to reduced variation in the resulting concentration of gene products (Cook et al. 1998). Finally, greater stability (longer half-life) of the RNA transcript and protein will also buffer against fluctuations in the rate of gene expression.

There is a considerable degree of robustness inherent in the sort of molecular control mechanisms that make up developmental systems. These systems continue to function normally even when subjected to major perturbations of their components, as has been demonstrated for gene regulatory networks (Little et al. 1999; von Dassow et al. 2000) and signal transduction processes regulating bacterial cell behavior (Barkai and Leibler 1997). Some specific types of gene regulatory circuits, such as gene autoregulation with negative feedback loops, have a stabilizing influence on behavior of the system (Becskei and Serrano 2000). Much remains to be done, however, before the dynamics of larger gene regulatory networks is fully understood, both in terms of further modeling studies (Smolen et al. 2000) and empirical studies in functional genomics (e.g., Wagner 2000).

Experimental evidence on molecular mechanisms that contribute to developmental stability is sparse. However, studies published in the last few years have applied new approaches that should be fruitful in producing information relevant to developmental stability. These studies should also point out systems in which the predictions about the macroscopic outcomes of these molecular processes can be tested empirically. A first example of this sort is the recent research on heat shock proteins.

The case of heat shock proteins

Heat shock proteins are molecular chaperones that recognize and bind to other proteins in denatured state, so that they can reestablish their proper conformation or be degraded. These proteins thereby help maintain functionality of other cellular protein components. Denaturing of proteins is especially serious when organisms are subjected to various kinds of stress, of which temperature stress is only one example, and heat shock proteins are therefore a crucial component of stress response (reviewed by Feder 1999; Feder and Hofmann 1999).

A possible role of heat shock proteins in maintaining developmental stability is suggested by an experimental study of one specific protein of this class, Hsp90, in *Drosophila melanogaster* (Rutherford and Lindquist 1998). Hsp90 is special among the heat-shock proteins in that it primarily interacts with proteins that are part of signal transduction pathways, maintaining their conformation ready for signaling interactions. Under conditions causing overall protein damage, however, Hsp90 is withdrawn from this specialized function and participates in the general stress response. In fly stocks heterozygous for mutant Hsp90 forms, Rutherford and Lindquist (1998) consistently found a small percentage of individuals with malformations in various body parts. Different lines of flies into which an Hsp90 mutation had been introduced tended to show different morphological defects, indicating a heritable basis associated with the genetic background. The strong and fairly sustained response to artificial selection for increased expression of defects suggested that these effects were polygenic.

In these experiments, the Hsp90 mutants revealed genetic variation in other genes which normally, in flies with the full level of Hsp90 activity, is not manifest in the phenotype. Given the normal role of Hsp90, interacting primarily with proteins of signaling pathways, it is likely that this genetic variation stems from genes connected to these pathways, and selection for increase of morphological defects would favor alleles that make signaling more reliant on Hsp90 for normal function. Failure of the mutant forms of Hsp90 in their role as molecular chaperones therefore may have led to insufficient activity in various parts of those signaling pathways, revealing the phenotypic effects of differences among allelic forms of those genes. The way in which this reduced signaling activity becomes manifest in the phenotype, with incomplete penetrance, may therefore be similar to a threshold response to the variable product concentration in the model of stochastic gene expression (Cook et al. 1998).

Moreover, increasing the temperature above the optimum for *Drosophila* development increased the incidence of morphological defects, enhancing the effects of Hsp90 mutants (Rutherford and Lindquist 1998). It is possible that this increase with temperature reflects the degree to which the functional Hsp90 present in the cells is diverted from its specialized role in supporting signaling pathways and used as part of the general stress response. Rutherford and Lindquist (1998) hypothesize that under stressful conditions, genetic variation normally concealed by Hsp90 activity could become manifest and subject to natural selection, increasing the potential for evolutionary change (see also Wagner et al. 1999).

In the context of developmental stability, on the other hand, this relation of Hsp90 to the occurrence of phenotypic variation also offers a possible explanation for the connection between FA and stress (e.g., Parsons 1990; Graham et al. 1993b). The function of heat shock proteins as molecular chaperones coping with denatured proteins that may be present as a consequence of stress and the up-regulation of these proteins as a direct response to stress (e.g., Feder and Hofmann 1999) suggest themselves as one example for cellular mechanisms providing developmental stability. However, this possibility requires further investigation through

theoretical modeling as well as experimental study. Moreover, there are many other mechanisms, working at all scales from the molecular to whole-organism levels, which also contribute to developmental stability.

Conclusions: towards realism and explanatory value of models

In this chapter, I have reviewed ideas and models regarding the developmental processes that form the basis of developmental instability. Modern developmental biology has much to offer to studies in this field, and should make it possible to add experimental methods to the mostly correlative and observational approaches that have dominated the literature so far.

Among the few models available, here is a clear trend from more phenomenological and descriptive models toward more process-oriented ones. So far, the spectrum of models spans from those that deliberately abstain from any mechanistic basis (Leung and Forbes 1997) through models of nonlinear dynamics (Graham et al. 1993a; Van Dongen et al. 1999b) to a simple diffusion-threshold process including a genetic component (Klingenberg and Nijhout 1999). The qualitative comparisons of the model results with patterns found in empirical FA studies are encouraging, as they have revealed new explanations of observed phenomena and suggested specific tests.

However, there is still a long way to go. In the past several years, new models have been proposed for the molecular and cellular processes that are a possible basis for the developmental instability as it can be observed macroscopically for morphological traits (Ko 1992; Cook et al. 1998; McAdams and Arkin 1999; Fiering et al. 2000). This approach is promising to make the study of developmental instability more explicitly mechanistic, and thereby compatible with the genetic and molecular approaches of current developmental biology. This mechanistic approach is also giving more specific meanings to some of the concepts used in traditional FA studies, making them more amenable to experimental and quantitative study, and is therefore leading to further development of the underlying theory.

I am confident that combining the conceptual background and biometric methods of FA studies with the experimental protocols and mechanistic approach of developmental biology will advance the understanding of developmental instability and of morphological traits in general.

Acknowledgments

I thank Michael Akam, Ricardo Azevedo, John Graham, Benedikt Hallgrímsson, Larry Leamy, Fred Nijhout, and Michal Polak for their helpful comments on an earlier version of this chapter. Financial support was provided by the Human Frontier Science Program.

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Figures and legends

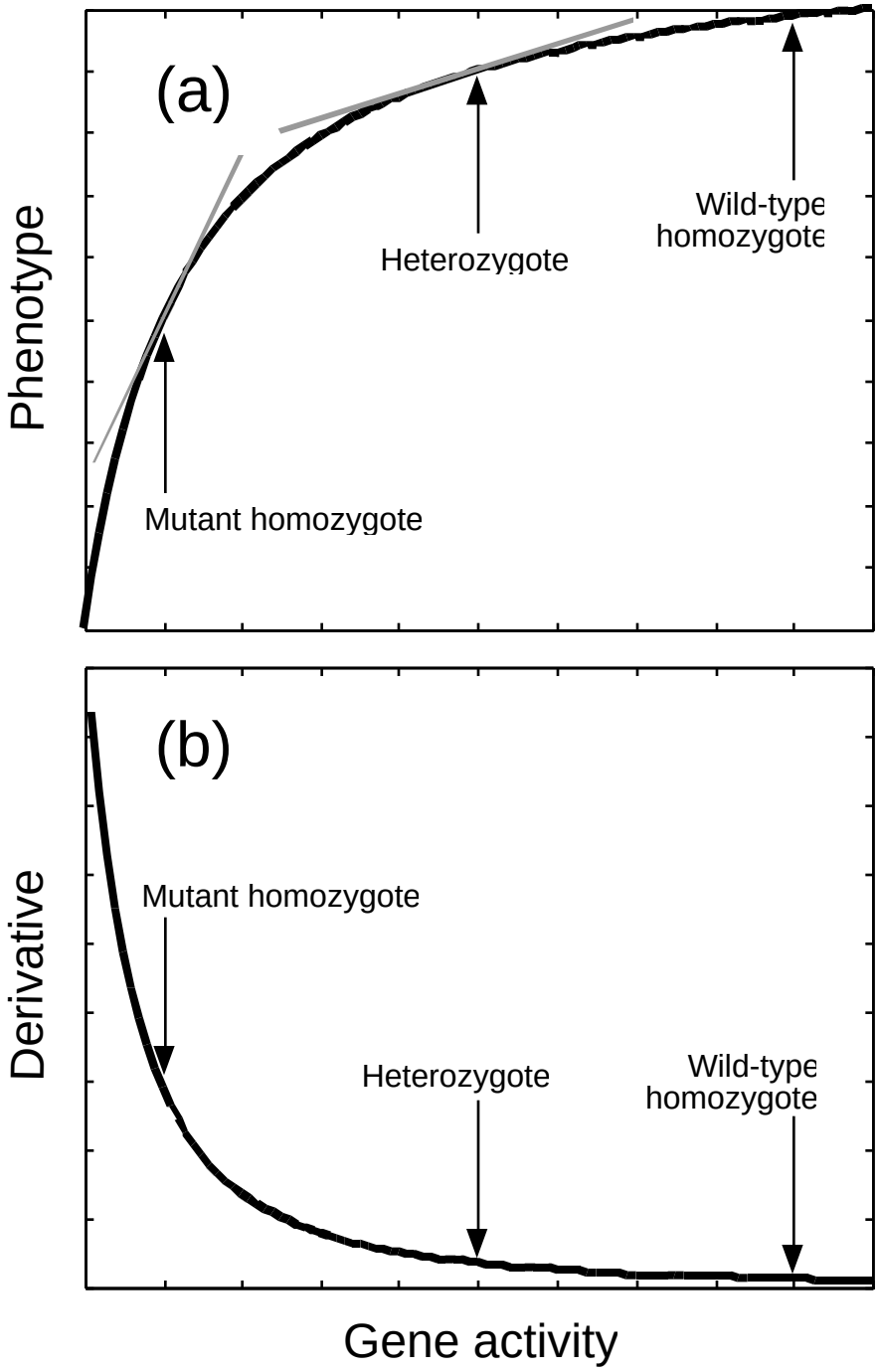

FIG. 1. Nonlinear developmental mapping. In this example, the phenotype is metabolic flux through a multi-enzyme pathway, and gene activity is the activity level of the one enzyme in the system whose activity is allowed to vary (Kacser and Burns 1981), but the same reasoning applies to a wide range of models that result in similar "diminishing-returns" curves. (a) Plot of the phenotypic value as a function of gene activity. The slope of the curve at the gene activity level of a particular genotype is a measure of the system's sensitivity to small perturbations in

gene activity, and thus of developmental instability. The curvature of the plot indicates dominance with respect to the phenotype. (b) The derivative of the curve in (a) presents these sensitivity of the developmental system as a function of gene activity. Notice that there is dominance with respect to the derivative. Modified from Klingenberg and Nijhout (1999), © Society for the Study of Evolution.

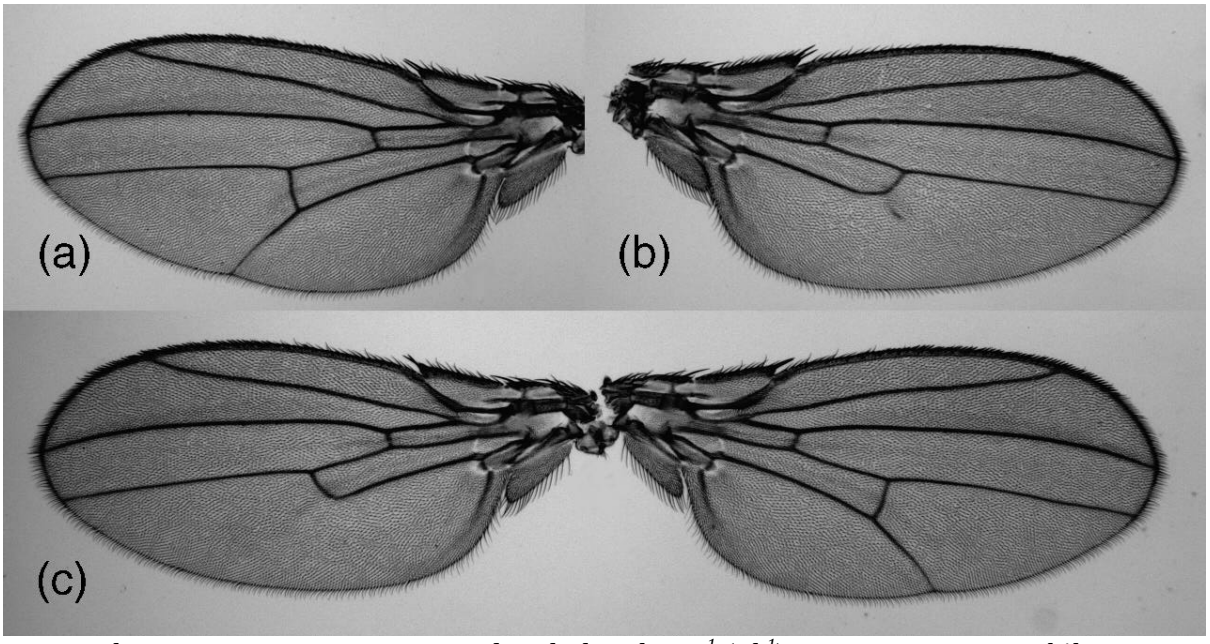

FIG. 2. Phenotypic variation associated with the *abrupt*^{1} (*ab*^{1}) mutation in *Drosophila melanogaster*. (a) Wild-type wing. (b) Strong *ab*^{1} phenotype, in which the fifth longitudinal (L5) vein is shortened to the intersection with the posterior crossvein. Weaker phenotypes are missing shorter pieces of the L5 vein, and do not affect the position of the posterior crossvein. (c) The two wings of a single fly from an *ab*^{1} mutant stock. The left wing shows a strong *abrupt* phenotype, while the right wing is effectively wild-type.