

Canalization in evolutionary genetics: a stabilizing theory?

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“The stability of the morphogenetic system is destroyed (rendered labile) due either to variation in environmental factors or to mutation. On the other hand, in the course of evolution stability is reestablished by the continuous action of stabilizing selection. Stabilizing selection produces a stable form by creating a regulating apparatus. This protects normal morphogenesis against possible disturbances by chance variation in the external environment and also against small variations in internal factors (i.e. mutations). In this case, natural selection is based upon the selective advantage of the norm (often the new norm) over any deviations from it.”

I.I. Schmalhausen, 1949
(p 79 of 1986 reprinted edition).

Summary

Canalization is an elusive concept. The notion that biological systems ought to evolve to a state of higher stability against mutational and environmental perturbations seems simple enough, but has been exceedingly difficult to prove. Part of the problem has been the lack of a definition of canalization that incorporates an evolutionary genetic perspective and provides a framework for both mathematical and empirical study. After briefly reviewing the importance of canalization in studies of evolution and development, we aim, with this essay, to outline a research program that builds upon the definition of canalization as the reduction in variability of a trait, and uses molecular genetic approaches to shed light on the problems of canalization. *BioEssays* 22:372–380, 2000.

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Introduction

The concept of canalization is viewed differently by developmental and evolutionary biologists. Wilkins defines canalization as “the stabilization of developmental pathways by multiple genetic factors within the genome, a form of genetic buffering”.⁽¹⁾ This notion builds firmly on the tradition of embryologists such as Schmalhausen⁽²⁾ and Waddington⁽³⁾ who emphasized the amazing stability of complex developmental processes. There is no doubt that such buffering exists, that there must be a variety of fascinating biochemical

mechanisms that produce it, and that it has implications for both developmental and evolutionary biology.⁽⁴⁾ However, evolutionary geneticists have devised a more limited meaning for the term canalization. Whereas buffering refers to keeping a trait constant and hence to low variance about the mean, the associated evolutionary questions are whether or not traits can evolve such that they become less likely to vary, and if so, whether particular ones have done so, and eventually to address how this has occurred. In other words, for the evolutionary biologist, canalization is genetic buffering that has evolved under natural selection in order to stabilize the phenotype.

In this essay, we begin by addressing why developmentalists should care about the phenomenon at all and then move on to discuss some of the problems for evolutionary theory that are raised by the existence of canalization. We then present several lines of evidence for, and summarize recent mathematical treatments of, the evolution of stability in the face of environmental and genetic sources of variation. This leads us to a discussion of emerging methods for the detection of canalization and of their potential to shed light on the mechanisms by which developmental stability is achieved. The aims of this essay are to raise awareness of the issues and to point out some directions for future research into the developmental and evolutionary causes of morphological homeostasis.

Canalization in development and evolution

Developmental biologists working at the interface with evolution are confronted with the phenomenon of canalization in many contexts, some of which are hinted at in Table 1. An important one is in the analysis of evolutionary rates. The evolvability of traits, which refers to their capacity to evolve,⁽⁵⁾ is affected by canalization in a variety of ways. Most obviously, any process that reduces the level of expressed variation of a trait reduces the capacity for evolution of the

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TABLE 1. Problems and Questions Associated with Canalization**A. Problems of interest**

Have developmental pathways evolved specifically to buffer the development of traits?
 If so, has this been mainly in response to stabilization of selection on environmental or genetic sources of variation?
 What are the consequences of canalization for the evolvability of traits?
 Does canalization actually increase the rate of divergence of developmental pathways, in the absence of morphological divergence?
 Does canalization contribute to the discreteness of cell type determination?
 Does redundancy constrain or promote the evolution of canalization?
 Are there multiple different mechanisms by which canalization can be achieved?

B. Research questions

How can canalization, as opposed to general buffering, be detected?
 Under what circumstances will canalization evolve?
 Can QTL analysis identify genes that contribute to canalization?
 How does canalization affect the distribution of molecular genetic variation at different levels of genetic hierarchies or regulatory genes?

trait, since the rate of evolution under natural selection is proportional to the amount of additive genetic variance.⁽⁶⁾ In the short term, canalization will tend to reduce the evolvability of affected traits and persistent canalization may contribute to macro-evolutionary stasis. On the other hand, it can be argued that canalization might increase the potential for evolutionary divergence. This is because the reduction of the effects of new mutations caused by canalization can allow a build-up of what is sometimes referred to as hidden genetic variation. If the expression of genetic variation is prevented, selection does not “see” the variation, which allows the accumulation of genetic differences. If the canalizing system then breaks down, either as a result of a change in selection pressure under different ecological circumstances, or after admixture of new variation, the genetic system will be poised for more rapid change than might otherwise be expected to occur.⁽⁷⁾ Even in the absence of morphological change, the accumulation of selectively neutral variation in canalized pathways is expected to increase the rate of divergence of the underlying genetic and developmental pathways,^(8,9) perhaps contributing to the evolution of hybrid incompatibility. Furthermore, if canalization suppresses the expression of deleterious side effects of adaptive mutations, it may directly increase the rate of evolution.^(10,11) Ascertainment of the prevalence and strength of canalization is thus a fundamental challenge in the study of evolution and development.

Independent of this context, canalization is relevant to the understanding of a wide variety of problems in developmental biology, from the discreteness of cell types to the symmetry of paired appendages. The genetic interactions within and among pathways that determine cellular identities have evolved not just to form particular developmental patterns, but also to ensure that they are stably maintained and well buffered.⁽⁴⁾ Just as canalization is hypothesized to contribute to the stability of visible quantitative traits, it could

make a contribution to the stability of decisions that are made at the cellular level, including commitment to differentiation and proliferation. In fact, the discreteness of cell types was the phenomenon that originally led Waddington to develop his concept of canalization.⁽³⁾ Most of the current approaches to these processes are molecular genetic, and mathematical canalization theory is unlikely to be adopted explicitly in the near future to address them. As genomic approaches generate vast pools of data on comparative gene expression levels and genetic divergence in developmental pathways, however, the mechanisms by which the low variance associated with many aspects of morphogenesis has evolved come into the reach of experimental and theoretical research.

Quantitative geneticists studying morphology are also unavoidably drawn to canalization, since the best-described developmental genetic pathways tend to regulate pattern formation mechanisms that are slowly evolving. The genes that are involved in processes such as segmentation, neurogenesis, and appendage morphogenesis are as susceptible to mutation accumulation as any other genes but little is known about the effects of natural variation in these genes. Is selection so strong that it removes all variation in developmental genes, or have genetic systems evolved so that they can effectively hide the effects of molecular variation? If the latter is the case (as canalization theory predicts), how much variation can accumulate without undermining the stability of the character, under what circumstances can this occur, and what are the consequences for evolvability?

The need for a formal definition of canalization

Buffering is a fundamental aspect of development. There are likely to be many molecular mechanisms involved, including genetic redundancy,⁽¹²⁾ feedback regulation,⁽¹³⁾ and coop-

erative biochemical interactions.⁽¹⁴⁾ These are more phenomenological descriptions, however, than quantitative explanations of buffering. We simply do not know in detail how the outputs of signal transduction pathways are kept within defined limits despite complex series of variable inputs, or why cell types are discrete, or how the parallel development of both sides of each body is coordinated in time and space. Important as such questions are, it is also true that even a complete mechanistic description of developmental buffering would not address the question of how and why the buffering originated in evolution. Given an optimal phenotype, selection ought to favor the evolution of genetic systems that tend to produce individuals close to the optimum but this is a qualitative, not a rigorous argument. Is the evolution of buffering inevitable? Are there constraints on how well buffered pathways can become? How strong must stabilizing selection be to produce buffering?

Still more fundamentally, we can ask whether the buffering of the focal character “evolved” at all. Every cellular and organismal property is an evolved trait, but evolutionary biology is concerned with identifying the particular level of organization at which it first evolved. For example, the function of the nervous system depends on the electric excitability of cell membranes. Obviously this, in itself, does not imply that membrane excitability evolved for the purpose of nervous system function. In fact, membrane excitability is a much older phenomenon, already present in single celled protists. In general, the current functional use of a biological mechanism or structure is often not the reason it originated in phylogeny. Furthermore, the mechanisms that maintain traits are not always directly related to the origins of those traits. All land mammals have four limbs, reflecting their derivation from a common ancestor in which the developmental control of this type of body plan was fixed. Whether the absence of six-limbed mammals is a function of persistent selection against this number, or simply a consequence of a mechanistic or genetic constraint that prevents the development of functional ectopic limbs⁽¹⁵⁾ is, however, a legitimate question for evolutionary biologists studying the stability of morphology.

It should also be appreciated that the utility of a concept to evolutionary theory increases when it can be incorporated into mathematical population genetic models. One of the most important parameters in contemporary population genetics is the mutational variance,⁽¹⁶⁾ which is an expression of the amount of new genetic variation produced by mutation in each generation relative to the amount of environmental variance for that character. The amount of variation that can be maintained in populations, the heritability of traits, and the potential for evolution are all functions to some extent of the mutational variance. For most traits, the mutational variance is of the order of 10^{-3} , which means that, starting from a single clone, it takes about one thousand generations to generate as

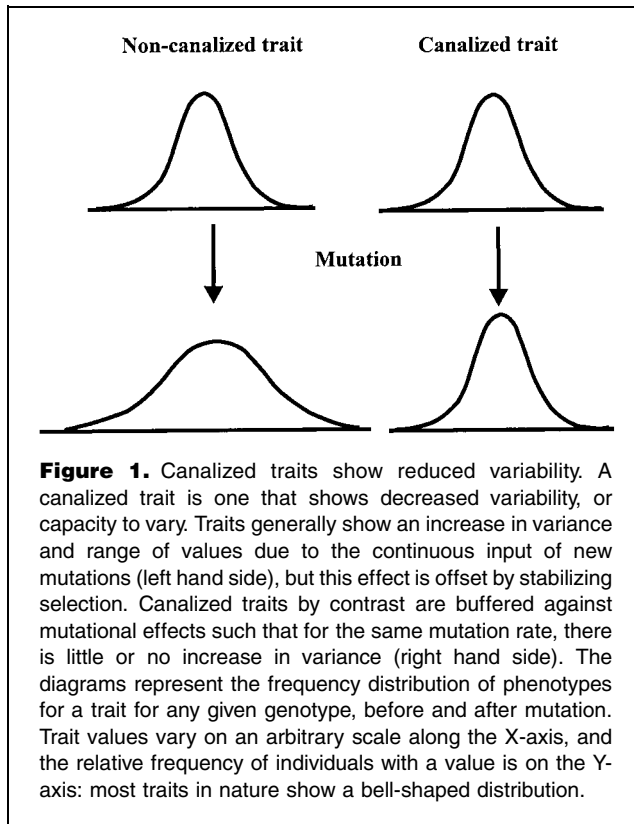
much genetic variation as the amount of variation due to nongenetic factors.⁽¹⁷⁾ If this parameter is itself variable, then genetic mechanisms that regulate the evolvability of traits must exist. The evolution of these genetic mechanisms is the true subject of research on canalization.

Canalization as a reduction in variability

Another way to express this is to define the process of canalization as any genetic change that results in a state of reduced variability of a trait.⁽⁵⁾ Variability is the propensity to vary, as opposed to the actual level of variation, much as power refers to the propensity to accelerate rather than the actual rate of acceleration. Variability is measured most directly as the mutational variance but as explained below this is not trivial to measure, hence, in practice it is generally easier to measure some associated parameter. Typically, this will be a reduction in the observed variance of a trait, though it must be borne in mind that simple changes in allele frequency can also affect variance. For example, if all of the variance in a trait is due to the segregation of two different alleles of a single locus, and one of those alleles disappears from the population, then the genetic variance will drop to zero but the variability, an intrinsic property, will not have changed. Any new mutation will change the variance of the trait, on average, by the same amount as it would have, had both of the original alleles been still present. A canalized trait is one in which the effects of new mutations are reduced such that the tendency (power) to vary is held in check.

The second practical problem with this definition of canalization is that the term “reduction” refers to some alternative state. If we are discussing the process of canalization, this alternative state is unambiguously the prior condition: canalizing selection reduces the variability at time t relative to that observed at time $t-1$. Happily, for population genetic modeling, this presents few difficulties. Empirically, however, if we are trying to infer whether a trait is already canalized, we need to be able to show that it has lower variability than is observed either under different conditions or under similar conditions in a different but related species.

Definition of canalization as a reduction of variability suggests that the major force responsible for the evolution of canalization will be stabilizing selection.^(18–21) This occurs when selection acts against individuals with extreme phenotypes on either side of some optimum. The immediate consequence of stabilizing selection is removal from the population of alleles that cause sub-optimal phenotypes.⁽²²⁾ As mentioned above, this reduces variance as a result of changes in allele frequencies but it is not sufficient to reduce variability. The term canalizing selection refers to the different process by means of which the genetic architecture of the trait evolves so that it is less likely to produce extreme individuals. This, in turn, is reflected in a reduction of the



average effects of new mutations, namely reduced variability, as diagrammed in Fig. 1. Stabilizing selection is a prerequisite for canalizing selection, but, crucially, it is not sufficient for it. Indeed, from a theoretical standpoint, it is not at all clear under which conditions canalization will evolve.

The reason why stabilizing selection is not sufficient for canalization is that the latter involves a delicate balance of forces that may not commonly occur. To see this, consider the following analogy. If you buy an old home, it will tend to be drafty and poorly insulated. You can do various things to increase the energy efficiency, such as putting in double glazed windows, buying better quality insulation, and making sure that doors close tightly. These adjustments may be more expensive, however, than just putting up with the high energy bills, or there may simply not be competent contractors or suppliers of the necessary technology available. Similarly, in trying to make a genetic pathway better buffered, the selective forces may not be great enough to achieve this, or the biochemical mechanisms may not be present, or if they are present they may have deleterious side effects which prevent their evolution. In general, canalization will only evolve given particular parameters of the existing variance, the deleterious effects of new mutations, and the intensity of stabilizing selection.⁽¹⁸⁾ These are not easily measured but often are likely to result in a situation where

genetic drift is as strong as natural selection in favor of canalization. In other words, saying that canalization *might* evolve is not the same as saying that it does.

Canalization against environmental and genetic factors

It is also useful to identify the source of variation for a trait in order to explain what canalization is actually buffering. It turns out that both the environmental and the genetic components of variation can be buffered, and that the genetic consequences are quite different.

For canalization against environmental variation ("environmental canalization"), the analysis is fairly straightforward. Any genetic changes that tend to reduce the sensitivity of an optimized trait to developmental noise will tend to be favored by canalizing selection.^(18–20) The classical example is fluctuating asymmetry associated with the evolution of insecticide resistance in blowflies.⁽²³⁾ Since both sides of an animal develop independently there is always some difference between them, for example in the number of bristles. The degree of asymmetry often increases in stressed organisms, as for example during the evolution of resistance to diazinon in the blowfly, but returns to lower levels when the stress is removed. Davis et al. have recently demonstrated⁽²⁴⁾ that the reduced asymmetry in bristle numbers in diazinon-resistant blowflies is associated with an increase in frequency of what appears to be an allele of the *Notch* gene. Whether this genotypic change was due to canalizing selection or to a correlated response to selection on some other trait, this case clearly shows that reduction of the variability of a trait can evolve. It is also a particularly pleasing example given the mechanistic connection between *Notch* function and bristle development.⁽²⁵⁾

By extension, it is likely that many traits are buffered against environmental factors as a result of canalizing selection. Wagner et al. argue that the only limit to the complete reduction of environmental variance is in fact mutation-selection balance.⁽¹⁸⁾ That is to say, the mutation rate will always be too high to remove all alleles that cause environmental sensitivity, and hence there will be a canalization limit. Evidence for such a limit comes from a classic artificial selection experiment performed by Prout.⁽²⁶⁾ When he selected for lines of *Drosophila* with either slow or fast developmental rates, the variance in developmental rates increased, indicating that genes can affect how much environmental variance there is in a population. By contrast, when he applied stabilizing selection to keep the developmental rate constant, genetic differences between lines were reduced but the level of environmental variance remained constant, indicating that this was already at its lowest selectable level in the starting population.

The role of environmental stress in the evolution of developmental buffering is an area of particular current

interest. Following Waddington,⁽³⁾ Eshel and Matessi⁽²⁷⁾ have argued that any increase in phenotypic variance caused by general disruption of morphogenesis when an organism develops in an extreme environment (for example, high temperature or low humidity) will be sufficiently deleterious to impose an additional canalizing selection pressure to favor the generation of phenotypes that are optimal in the most common environment. They further suggest that it would be of advantage for canalizing systems if they break down when conditions become too extreme, as this would favor the establishment of the rare newly adapted forms. This raises the question of whether buffering only operates within definable limits set by the physiology of each trait. In this regard, Rutherford and Lindquist's⁽²⁸⁾ demonstration that mutation of one of the major heat shock protein loci in *Drosophila* (*Hsp83*) destabilizes numerous developmental processes encourages the hope that a molecular understanding of developmental stability may be within reach. *Hsp83* mutant flies show malformations of each of the appendages but whether it is the wing, eye or leg that is most affected depends on the wild-type genetic background. Hence, there is certainly genetic variation available that could affect the response to extreme environmental perturbation.

For canalization against genetic variation ("genetic canalization"), the picture is far less clear.⁽¹⁸⁾ This term refers to the buffering of developmental pathways against the tendency of new alleles to make nonoptimal phenotypes. There are a number of nice examples of genetic canalization, none better than the effect of the *Tabby* mutation on whisker number in mice.⁽²⁹⁾ Almost all mice have 19 secondary vibrissae on each side of the snout, with the occasional variants having 18 or 20 such whiskers, and most of this variation is not heritable. In the presence of the *Tabby* mutation, the mean is reduced to 12, and the variance increases dramatically such that the range of values is 8–16 whiskers. Much of this variation is heritable as shown by the fact that it responds to artificial selection. This increase in

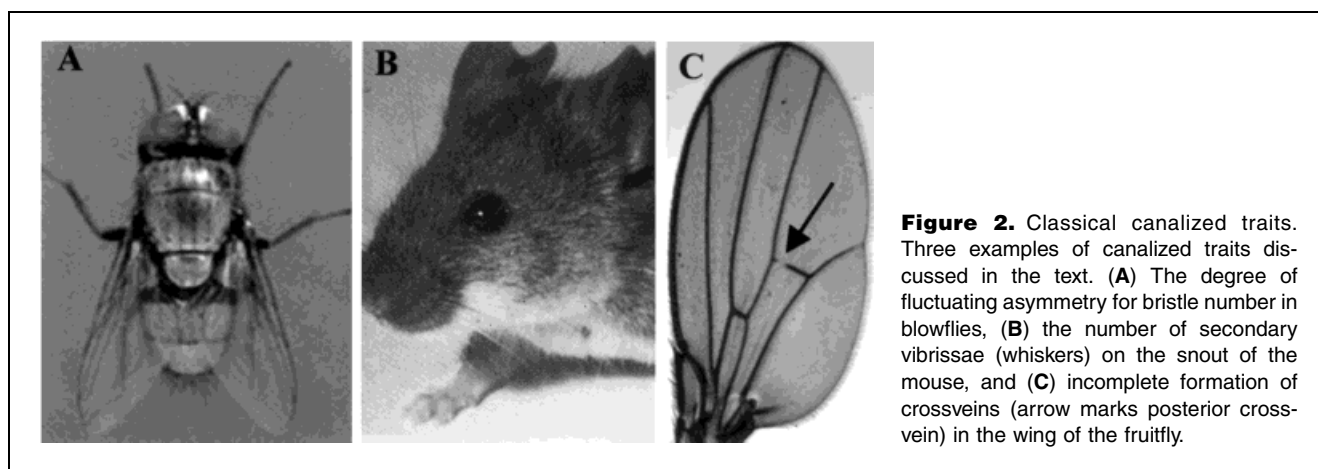
variance is interpreted as implying that the wild-type state has lower variability, and hence is canalized. Several other examples have been well studied in *Drosophila*, including bristle numbers and cross-vein formation on the wings, as reviewed by Scharloo (Fig. 2).⁽³⁰⁾

The conditions under which genetic canalization evolves are likely to be highly restricted, according to a recent theoretical analysis.⁽¹⁸⁾ Without going into the mathematics, at least one restriction must be confronted. This is that natural selection can only reduce the variability of a trait by selecting on so-called "gene interaction effects". That is to say, canalization results from changes in the influence of one gene on the effects of variation at another locus. These effects are only present if there is allelic variation at both loci, which in turn means that the amount of genetic variation in the population is of critical importance for the potential to evolve canalization. A consequence is the paradox that traits subject to the strongest stabilizing selection may, in fact, be the least likely to evolve canalization. This is because selection only acts on genetic variation, but if stabilizing selection is intense enough, then it will remove the source of variance that canalizing selection would act upon.

These analyses leave us with the disturbing conclusion that genetic canalization may be too difficult to attain directly by natural selection to account for the pervasive buffering of genetic pathways.^(18,20) Clearly, we need broadened theoretical treatment, and more rigorous empirical studies, to address the discontinuity between theory and observation.

Before discussing such research, we suggest two possible solutions to this problem. One solution may be that genetic canalization results from selection against environmental variation and is just an incidental side effect of the evolution of environmental canalization. In the language of population genetics, genetic canalization may be a correlated response to selection for environmental canalization.

The second, complementary, possibility requires that a distinction be drawn between "evolved" and "intrinsic"



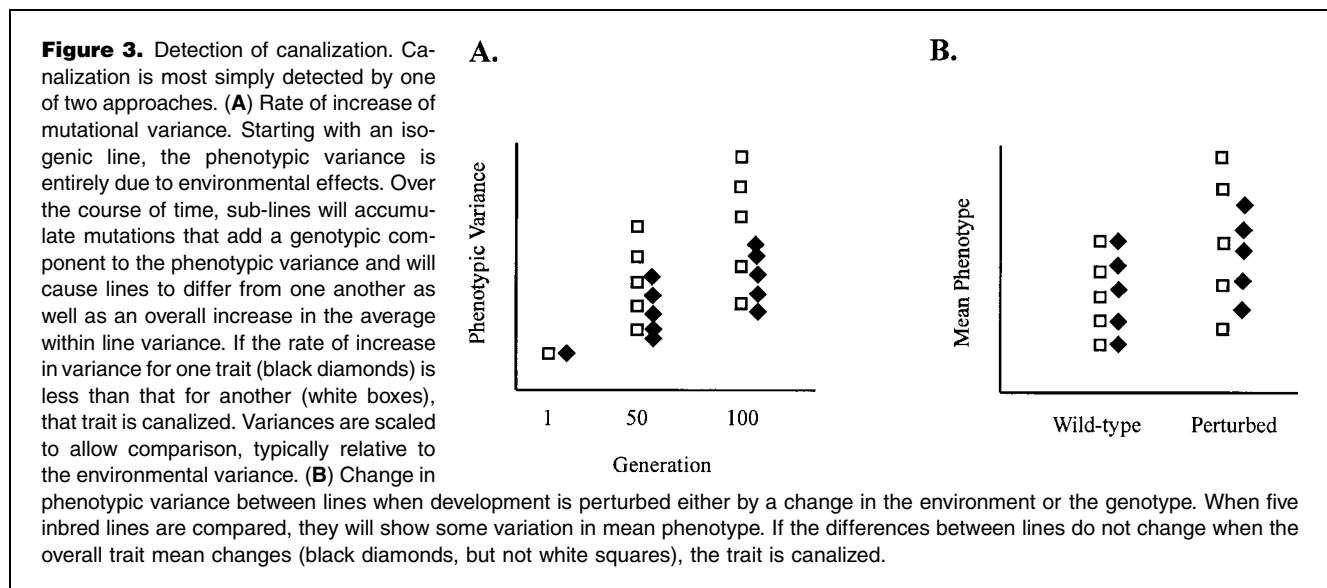
canalization. Returning to the analogy of the drafty house, suppose now that we have the alternative of buying a contemporary home complete with solar heating, computerized energy control, and high-tech materials. Relative to the old home, this new model is much better insulated, and in fact all homes built in the future with the new technology will be “canalized” in this way. The new technology was designed (evolved) to do the same thing as the ad hoc modifications that had to be made to the old home, but the changes are built into the home rather than being added on. In a similar way, we envisage that genetic mechanisms evolved in the past that predispose pathways to be canalized but that these mechanisms need not be continually shaped by canalizing selection on the particular pathway in which the mechanism is deployed. They are by now intrinsic. For example, if cooperative DNA binding of transcription factors is a method for achieving threshold-dependent transcriptional regulation⁽¹⁴⁾ and this in turn leads to buffered genetic switches, then any switch built with that “technology” will be intrinsically canalized. If the buffering mechanism is redundancy of gene function resulting from gene duplication,^(12,13) it is even possible that positive selection for stability played no role establishing the apparently canalized state. If this is the case, then it will be important to devise experimental strategies that can distinguish evolved from intrinsic canalization.

The detection of canalization

Following from our definition of canalization as a state of reduced variability, the most direct way to detect it is to measure the mutational variance of the particular trait and compare this with other mutational variances⁽³¹⁾ (Fig. 3A). There has recently been an upsurge of interest in the

measurement of mutational variance so that despite the technical difficulty and length of time required to get reliable estimates, sufficient data are now available for comparative purposes.⁽³²⁾ Nevertheless, there are several drawbacks that make this approach impractical as a general method. First, the measurement error of plus or minus an order of magnitude could well be as great as the difference canalizing selection might make. Second, there is some debate as to whether the use of isogenic lines as starting populations (which is done to remove the complication of natural selection during the mutation accumulation experiment) introduces a sizeable bias that renders any results impossible to extrapolate to outbred populations.⁽³³⁾ Third, there is usually no negative control, in the sense that any estimate of mutational variance is a point estimate that cannot be compared with another estimate from an earlier noncanalized population. Fourth, and probably most problematic, it is likely that variability is affected by numerous factors other than the level of canalization resulting from modification of the genetic architecture. Primary among these is the number of genes affecting a given trait, and hence the complexity of the trait.⁽³⁴⁾ Comparing variabilities in ‘fitness’ with ‘wing shape’ is like comparing apples with oranges.

Most studies of canalization have actually involved monitoring the response of a trait to some type of perturbation, be it environmental or genetic.⁽³⁰⁾ Rendel published an influential study of bristle numbers and wing veins in *Drosophila*,⁽³⁵⁾ showing that genotypes do not map onto phenotypes in a simple manner, which led him to develop a theory of canalization based on threshold-dependent responses to biochemical variables. This work has been criticized on the basis that in some cases it is not clear that



the particular trait being examined is the result of a single genetic pathway,⁽³⁶⁾ and, more recently, on the basis that we are left far short of a molecular genetic understanding of the phenomenon of reduced variation.⁽³⁷⁾

A promising extension of classical genetic strategies is to examine the effects of introgression of mutations into a panel of inbred lines, and then to document the change in variance (Fig. 3B). Moreno⁽³⁸⁾ reported a dramatic increase in variance of *Drosophila* bristle number in *extramacrochetae* backgrounds relative to wild-type backgrounds, consistent with the canalization hypothesis. Similarly, Polaczyk et al.⁽³⁹⁾ have shown that photoreceptor determination in the *Drosophila* eye is canalized, since a normally uniform process becomes highly variable in the presence of mutations that increase the tyrosine kinase receptor activity that initiates R7 photoreceptor determination. In this case, the effects of two different types of mutation are only weakly correlated across genetic backgrounds, suggesting that different alleles contribute to the buffering of different branches of the genetic pathway. By contrast, introgression of an *Ultrabithorax* mutation into a panel of inbred lines provided evidence for extensive variation for genetic interactions affecting haltere size but since there was no change in the variance in wild-type and mutant backgrounds it is not clear that this stable trait is actually canalized.⁽⁸⁾ This may be an example of the paradox that the most strongly selected traits cannot be genetically canalized.⁽¹⁸⁾

Another strategy for detecting canalization is to compare the stability of a trait with an a priori expectation, given information about the molecular mechanisms of the development of the character. To date, this has only been attempted in relation to a relatively simple phenotype, namely RNA secondary structure.⁽⁴⁰⁾ The demonstration that the secondary structure of a viral RNA genome is more stable to mutations than is a randomly chosen sequence that folds into the same structure shows that the virus has evolved to a state of increased mutational stability. It does not prove that selection acted directly on the mutational stability of the RNA secondary structure, the robustness of which may be a side effect of selection for a mechanistically linked process, increased thermodynamic stability, which is the molecular analogue of environmental variability.⁽⁴¹⁾ These studies provide the first hint that “genetic canalization” (mutational robustness in this case) can in fact evolve as a correlated response to selection for “environmental canalization” (thermodynamic stability) as predicted by population genetic models.⁽¹⁸⁾

From a molecular biological point of view, it will be just as important to study the mechanisms that produce developmental buffering. These range from redundant gene activity through DNA methylation to biophysical interactions. Clearly, this type of approach is very different from the population/quantitative genetic strategy discussed in this essay, though

it would be most satisfying if the two intellectual approaches eventually fuse. An obvious way in which this might occur is in distinguishing between evolved and intrinsic cases of canalization. Knowing the buffering mechanism—say, use of the MAP kinase cassette in photoreceptor determination—might suggest whether or not stabilizing selection directly on the trait is really responsible for the observed stability of the trait. Similarly, the finding that genetic redundancy contributes to a reduction in variability⁽¹²⁾ should influence our modeling of the population genetic processes responsible for the phenomenon.

Finally, it is becoming possible to directly dissect canalization by using genetic strategies analogous to those used to dissect any other quantitative trait. A remarkable example is provided by the demonstration of the existence of quantitative trait loci (QTLs) affecting the fluctuating asymmetry of skull morphology seen in the progeny of a cross between two inbred lines of mice.⁽⁴²⁾ Similarly, the identification of *Notch* as a candidate gene for the reduction in fluctuating asymmetry in diazinon resistant flies followed a recombination mapping protocol.⁽²⁴⁾ More generally, as argued in relation to the mapping of a naturally occurring modifier of the *Ultrabithorax* mutation in *Drosophila*,⁽⁴³⁾ it will be possible to identify genes involved in the buffering of traits by identifying QTLs for the trait itself, and then asking whether these factors interact additively, or show epistasis as expected of canalized systems.

Conclusions

The concept of canalization is often identified with the buffering of development, but, with respect to evolution, has a more specific meaning. The significance of canalization is that it reduces the variability of traits and, hence, their capacity to respond to selection or to diverge by genetic drift. Understanding canalization thus requires appreciation of the biochemical and/or genetic mechanisms involved, as well as the population genetic dynamics of alleles that regulate the variance of a trait. Recent theoretical models of canalization have shown how important it is to distinguish between environmental and genetic canalization, because these modes make different predictions about the likelihood that canalization will evolve.^(18–20)

Disturbingly, the available mathematical treatments suggest that the conditions under which genetic canalization will evolve are very limited.⁽¹⁸⁾ This is reminiscent of the debate about the evolution of dominance: population genetic theory does not predict that dominance should be pervasive, yet it is.⁽⁴⁴⁾ Since the vast majority of wild-type alleles are dominant, most new mutations must be recessive. Either these become dominant as they progress to fixation (due to selection on modifiers that affect their degree of dominance, although mathematical theory does not really support such a process,⁽⁴⁵⁾) or there are properties of the physiology of

TABLE 2. Modes of Canalization

Level	Type	Possible examples
Evolved	Genetic	Wing vein formation Organ size and shape
	Environmental	Insecticide resistance
Intrinsic	Apparent	<i>Drosophila</i> bristle number
	Biochemical	Ommatidial development
	Redundancy	Hybrid stability

gene function of favored alleles that make them intrinsically dominant.⁽⁴⁴⁾ A similar distinction may help to explain the apparent prevalence of canalization, hence our classification of canalization into evolved and intrinsic forms.

It is worth pointing out that there is very little information pertaining to how prevalent each mode of canalization is in nature but we offer the expanded classification in Table 2 as a spur to further research on the subject. Evolved canalization refers to that due immediately to canalizing selection on the trait, or in cases of “apparent canalization” to selection on some highly correlated pleiotropic traits, regulated by the same genes, that is more directly subject to stabilization of selection⁽⁴⁶⁾ (as for example, abdominal bristle numbers in *Drosophila*, which are thought to experience apparent stabilizing selection as a result of selection acting on a pleiotropic trait⁽⁴⁷⁾). Intrinsic canalization refers to regulatory mechanisms, which may themselves have evolved under canalizing selection at some time in the past, but which need not have been directly shaped by canalizing selection on the trait of interest.

Within the class of evolved genetic canalization, it may be worthwhile to distinguish a mode of canalization that has its roots in the genetic complexity and covariances among components that regulate the trait as discussed in a recent novel mathematical treatment by Sean Rice.⁽⁷⁾ He pointed out that wherever a phenotype is regulated by two or more pathways that interact in a nonlinear manner, it is possible for canalization to evolve. Suppose, for example, that the size of an appendage is a function of both cell size and cell number. Then there will generally be several combinations of the two parameters that give rise to identical sizes, and stabilization of selection will favor that combination which is associated with the lowest phenotypic variance. So long as the interactions between the two pathways that regulate cell size and number are not simply additive, there will be differences in the level of canalization of various optimal combinations of cell size and number. One consequence of this model is that a change in the trait optimum may allow the genetic system to evolve in different directions, say one increasing in cell number, the other increasing in cell size. Rice argues

cogently⁽⁷⁾ that such canalized systems are likely to be poised at a point where they are highly susceptible to divergence when the environment changes and offers this as an explanation for the “breaking of von Baer’s laws”, namely, genetic divergence between organisms with morphologically similar developmental processes.

Despite the fact that they have not yet had much of an impact on developmental biology, we propose that the methods of empirical research outlined in the previous section will help to untangle the various mechanisms that are responsible for canalization. Theoretical developments will also be required, particularly methods to analyze (i) the impact of pleiotropic genetic interactions on variability; (ii) selection on genotype-by-environment interactions; and (iii) the consequences of canalization. These phenomena obviously include both the capacity of genetic systems to maintain variation that can be unlocked when the system is perturbed, leading to changes in previously stable traits, and the constraints that are placed on morphological divergence by the existence of canalization. Just as important to understand is the capacity for genetic divergence in canalized systems, and its role in the evolution of hybrid inviability mechanism and also in speciation. As genetic analysis uncovers the loci that are responsible for quantitative variation, it will become possible to link theoretical and empirical studies at several levels of biological organization.

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