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A single mode of canalization

Colin D. Meiklejohn and Daniel L. Hartl

The evolution of mechanisms underlying the buffering of the phenotype against genetic and environmental influences has received much theoretical and experimental attention, yet many issues remain unresolved. Here, we consider the kinds of biological process that are likely to promote this buffering, or canalization, and the circumstances under which the evolution of these mechanisms will be favored. We conclude that evolution should produce a single mode of canalization that will buffer the phenotype against all kinds of perturbation, and that the major fitness benefit driving the fixation of canalizing alleles derives from a reduction in environmental influences on phenotypic variation.

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Biological development produces stereotyped outcomes, such as discrete tissue types and organs, with few intermediate forms. The term 'CANALIZATION' (see Glossary) was coined to describe this phenomenon of discrete developmental outputs [1,2], and has been extended to include the ability of such systems to withstand genetic or environmental perturbations. Canalization has historically been inferred from the observation of organisms under genetic or environmental conditions that result in an increased range of phenotypic variation. This variation is then shown to have a partially heritable basis, indicating the presence of genetic and environmental variation that was masked under normal conditions (Box 1). Several terms have been used to describe this property, such as HOMEORHESIS [3] and phenotypic or DEVELOPMENTAL BUFFERING or stability (the history and usage of these terms is reviewed in [4]). Here, we use these terms entirely synonymously in the following manner: a homeorhetic (or canalizing) allele is one that reduces the PHENOTYPIC VARIANCE of a trait across genetic backgrounds and environments relative to a nonhomeorhetic allele. Similarly, a canalized trait (or a trait exhibiting homeorhesis) is one that demonstrates a restricted range of variation across genetic backgrounds and environments relative to a noncanalized trait. Canalization is therefore recognized as a property of organisms that influences their variability, or their propensity to vary [5].

Although the evolutionary fate of mutations that contribute directly to phenotypic differences has been studied extensively, the evolution of alleles that constrain or promote phenotypic variability is less well understood. Several theoretical studies have recently looked at the subject of variability and evolution, focusing on the evolution of mutation rates [6] as well as on the evolution of canalization [7–9]. Within the neodarwinian framework, the causes of variation are independent of the consequences

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Box 1. Initial studies on canalization

Waddington [a] performed the first set of experiments that directly revealed the phenomenon of canalization. He began with a strain of *Drosophila melanogaster* that, when exposed to heat shock during pupation, sometimes partially or completely lacked the small crossvein between major veins four and five on the wing. Artificial selection for both increased and decreased frequency of the crossveinless phenotype was successful and, after 16 generations, 1–2% of flies in the crossveinless selected line, which had not been exposed to heat shock, nonetheless showed the crossveinless phenotype. Both the selection response and the manifestation of the phenotype in untreated individuals were hypothesized to be the result of phenotypically cryptic genetic variation that had been revealed by the environmental treatment.

Waddington repeated this experiment with a much more dramatic phenotype, the bithorax phenocopy [b], in which the third thoracic segment is transformed to resemble the second, in the most extreme cases, with a complete second set of wings in place of halteres. Individuals form the bithorax phenocopy in response to exposure to ether at a certain stage of development. Waddington again observed a response to selection for increased and decreased frequency and severity of the bithorax response, and again found individuals who manifested the bithorax phenotype even in the absence of ether treatment.

Rendel [c] extended this work with the *scute* mutation in *Drosophila*, which reduces the number of scutellar bristles from an invariant wild-type number of four to a variable range between zero and three. Artificial selection for fewer scutellar bristles resulted in a decrease in bristle number in wild-type siblings of *scute* flies, and selection for increased bristles produced wild-type siblings with more than four bristles. By analysing the proportion of the selected lines that comprised each bristle number class, he showed that the four scutellar class was the widest (Fig. I). Therefore, the *scute* mutation and artificial selection changed the amount of some underlying variable responsible for bristle formation, without altering dramatically the developmental process that transforms that variable into an actual bristle number, and which canalizes the phenotype about the wild-type state. In a later set of experiments, Rendel showed that he could also alter the homeorhetic process to canalize a two-bristle phenotype [d], demonstrating independent heritable variation for both bristle number and its canalization.

It is possible that the heat shock and ether employed by Waddington either shifted development outside the range of homeorhesis, as with *scute*, or that development was decanalized by transforming the sigmoidal relationship in Fig. I into a linear one; however, without analyses of the sort performed by Rendel, it is impossible to distinguish whether Waddington disrupted homeorhetic mechanisms, or simply moved a developmental process outside of their range.

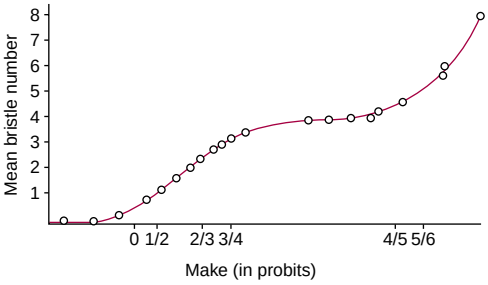

Fig. I. Canalization of bristle number in *Drosophila*. Artificial selection to increase or decrease bristle number in the presence of the *scute* mutation revealed that some bristle number classes are more resistant to selection than others. The abscissa refers to the width of each bristle number class in probits, a statistical measure of the proportion of the selected populations that remained in each class during the selection experiments. Lines indicate the boundaries between the different bristle number classes. The underlying variable promoting bristle formation was termed 'Make'. Both the *scute* mutation and artificial selection changed the amount of Make. That the underlying developmental process is canalized about four bristles can be seen by the fact that a much larger change in Make is required to move through the four bristle class than through any of the other classes (as seen by the width of the four bristle class). Reproduced, with permission, from [e].

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that the variation has for evolutionary change. Analogously, an understanding of the evolution of variability requires a distinction between the causes promoting changes in variability and the consequences of altered variability for further evolutionary change. Here, we consider the evolution of canalization with this distinction in mind.

We focus specifically on identifying molecular and developmental mechanisms that are likely to be homeorhetic, and under what conditions selection can promote the fixation of alleles with these properties. We argue that the major source of selection pressure for canalization results from the benefit gained by buffering the effects of environmental perturbations, but that canalization, once evolved, will act to buffer any and all sources of variation. Because of the paucity of data directly relevant to this problem, much of our argument is motivated by broader considerations of biological organization and evolutionary theory. Additionally, the diversity of biological systems and the contingency of the evolutionary process make it inevitable that exceptions to these rules exist; however, we remain confident in the generality of these conclusions.

Homeorhetic mechanisms are general

Many authors have considered whether homeorhetic mechanisms are general or specific with respect to the types of perturbation that they buffer. The most common consideration of this kind is whether a system that is buffered against genetic perturbations will also be insensitive to environmental perturbations, and vice versa. There has been argument in favor of this hypothesis [3,10–14], as well as the opposing claim that separate homeorhetic systems buffer against the two classes of perturbations [7,12,15]. Few experimental studies have addressed this issue directly, but the results consistently point in favor of the generality of homeorhesis.

First, expanding on the original work with the *scute* locus in *Drosophila*, Rendel and Sheldon selected for both increased and decreased canalization about a novel phenotype [16]. Selection for increased canalization resulted in an increase in the width of the selected class in probits (Box 1), a reduction in the phenotypic difference between males and females, and a decreased sensitivity of the phenotype to temperature [16].

Second, Stearns *et al.* [17] identified components of genetic and environmental canalization by considering

Box 2. Enzymes, dominance, and homeorhesis

It has long been recognized that loss-of-function mutations and mutations with large phenotypic effects are usually recessive [a]. To explain the dominance of the wild-type allele, Wright showed that the mathematical relationship between the metabolic flux across a step in a linear biochemical pathway and the activity of the enzyme catalysing that step is a hyperbola [a] (Fig. I). Such saturation kinetics result in a asymptote in the reaction velocity as the activity of the catalysing enzyme increases. This means that for enzymes whose activity is near or in the asymptotic region, small changes in activity will have negligible effects on the phenotype and fitness. In other words, the phenotype will be canalized against genetic or environmental perturbations that displace enzyme activity from its optimal level. This means that organisms may rather easily evolve canalization simply by increasing enzyme activity until it is saturating.

Linear metabolic pathways are even more robust to changes in the activity of any one enzyme in the pathway, because each enzyme contributes only fractionally to the control of the overall flux through the pathway [b]. The dependence of the flux on any given enzyme is a function of the rate constant for the reaction catalyzed by that enzyme, as well as the rate constants for all other enzymes in the pathway. If all enzymes in a pathway have the same control over the flux (i.e. a given change in activity for each enzyme results in the same change in flux through the pathway), then the dependence of the flux on any one enzyme decreases as the number of enzymes in the pathway increases. This was recognized by Kacser and Burns [c] as a feature producing inherent recessiveness of mutations affecting metabolic enzymes interacting in linear pathways.

Several experimental measurements on the relationship between enzyme activity and metabolic flux indicate that most enzymes are maintained in organisms at activities that are close to saturation (summarized in [d]). The degree of recessivity of lethal mutations as well as the equilibrium frequency of null alleles in natural populations of *Drosophila* also points to an asymptotic relationship between gene activity and fitness [d].

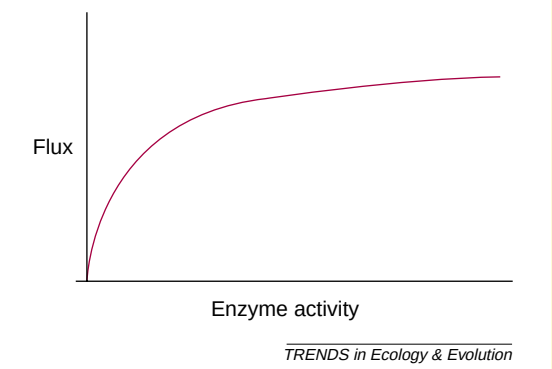

Fig. I. Saturation kinetics. The relationship between the activity of an enzyme and the reaction rate or metabolic flux through the step catalysed by that enzyme is hyperbolic. This can be seen by a simple example: assume a metabolic intermediate A is converted to a product B at a rate x that is directly proportional to the activity of enzyme X. Assume further that the rate of input of A is a, the rate of removal of A is k, and the rate of removal of B is j. Then $dA/dt = a - A(x + k)$ and $dB/dt = Ax - Bj$. At equilibrium, $dA/dt = dB/dt = 0$ and the flux through the pathway to molecule B is $ax/(jx + jk)$ which is hyperbolic with respect to x.

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the variance of traits between and within inbred lines, respectively. They observed that the phenotypes most strongly canalized against environmental perturbations are also those that are most strongly canalized with respect to genetic perturbations [17].

Third, work on early embryogenesis in *Drosophila* has shown that the *hunchback* gene shows less variation in its expression between embryos than does the expression of *bicoid*, the morphogen that activates *hunchback* [18]. At the same time, *hunchback* expression profiles are also less sensitive to temperature than are *bicoid* expression profiles. Whichever other factors are responsible for this increased precision in *hunchback* expression also confer greater buffering against environmental perturbations.

Finally, computational studies of RNA secondary structure indicate that mutational and environmental sensitivity of RNA sequences might be inherently correlated [19]. An RNA sequence can fold into a range of secondary structures, and spends a fraction of time in each structure that is dependent on the chemical free energy of that structure. The proportion of time that a sequence spends in its most stable (minimum free energy) structure is positively correlated with the proportion of single mutation neighbors that share the same minimum free energy structure [19]. Sequences that are robust to stochastic thermal fluctuations in their secondary structure are therefore also robust to mutations. Simplified modeling of protein structures suggests that a similar correlation might also exist for proteins [20]. It remains to be seen whether this congruence can be generalized to higher levels of biological organization.

A consideration of how canalization can occur also suggests the generality of buffering mechanisms. Most loss-of-function mutations are recessive, which implies that organisms are able to compensate for even a 50% reduction in gene activity, a large perturbation by any standard. This recessivity can be understood as an inherent feature of enzyme activity and metabolic pathways (Box 2). Most groups of functionally linked developmental genes probably do not act in linear pathways, but form more reticulate networks of interactions. Such networks might be expected to be even more robust not only to changes in gene dosage, but also to quantitative changes in the interactions between genes and gene products. Indeed, recent modeling approaches have shown that both the segment polarity and neurogenic networks in *Drosophila melanogaster* are highly robust to large changes in effective gene dosage, the strength of interactions and, to a lesser extent, to changes in network topology [21,22]. This list is undoubtedly a very small subset of the ways in which biochemical, cellular and developmental organization can canalize the phenotype. As long as environmental perturbations affect the amount or activity of gene products participating in metabolism and development in a manner that is qualitatively similar to gene mutations, they too will be buffered by these same mechanisms.

Natural selection should favor general buffering mechanisms

Even if alleles that confer a specific mode of canalization were common, there would be little opportunity for selection to favor such an allele over one with a more general homeorhetic capacity. This is because there is no aspect of an organism that is inherently and persistently vulnerable to genetic but not environmental perturbations, or vice versa. Organismal phenotype is determined by interactions between genotype and environment (as well by interactions among genes and among environmental effects). All aspects of organismic form and function are affected by the genotype to some extent, and allelic variation affecting quantitative traits is usually segregating in natural populations [23]. The prevalence of such variation is also shown by the observation that, in outcrossing species, few quantitative traits are resistant to change under artificial selection [24]. The variety of morphological and biochemical change that has resulted from artificial selection in domesticated animals and plants is further testament to the rich potential of genetic control of the phenotype. Because they influence the phenotype through their interactions with the genotype, forces that are external to the genome can create phenotypic variation in just as broad a range of traits as can the genotype. Thus, for a trait where the suppression of phenotypic variation is favored, an allele that constrains against both classes of variation should, over evolutionary time, always be favored over one that buffers only one class of perturbations. In conjunction with the scarcity of specific buffering mechanisms, this suggests that the fixation of a genetic or environment-specific homeorhetic mechanism should be rare indeed.

How does canalization evolve?

Evolution of a phenotype that reduces sensitivity to perturbations is facilitated by selection favoring a restricted range of the current variation within a population, or stabilizing selection, although other selection regimes might also select for canalization [25]. However, this insensitivity might also be an inevitable consequence of constructing an organism. Because development is a probabilistic process, natural selection must favor genotypes that produce a fit phenotype with high reproducibility (or selection for DEVELOPMENTAL STABILITY [4]). Because no organism develops in the absence of background genetic and environmental variation, this reproducibility necessarily includes robustness to these perturbations. Selection for developmental processes that successfully produce their target phenotype will therefore implicitly select for canalization. This relationship has been explicitly demonstrated *in silico* [26].

Thus, in the context of the present discussion, we extend stabilizing selection to include not only the condition of a most-fit intermediate phenotypic value, but also the more general requirement that the organism retains a coherent and harmonious integration of its processes of development, physiology and morphology. Under this view, the core developmental processes of most organisms are under stabilizing selection. This is supported by the fact that most large changes to developmental systems are deleterious, because mutations with visible phenotypic effects almost without exception produce a reduction in fitness [23,27], and also by numerous artificial selection experiments in which selected lines reach a plateau as a result of natural selection against the more extreme phenotypes [28]. We should therefore expect evolution to have produced canalized phenotypes frequently in nature, owing to the long-term effects of strong, pervasive selection for biological systems that are stable against perturbations away from optimal phenotypes. That canalization has been documented in many biological systems (reviewed in [11]) agrees with this expectation. Even in the case of adaptive PHENOTYPIC PLASTICITY, where selection favors a developmental system that maps environmental variation onto a specific range of phenotypic variation, reaction norms are tightly regulated to produce a very small subset of the possible phenotypes [29], and global constraint of the phenotype is still favored.

One of the most detailed examples of the association between stabilizing selection and canalization comes again from a computational study of RNA secondary structures. Wagner and Stadler [30] compared regions of the genomes of RNA viruses that are known to have a function associated with their secondary structure to regions that are inferred to have reduced or no constraints associated with their secondary structure (such as protein coding sequences). They found that the former class of sequences form structures that are less sensitive to mutations than are the latter. This robustness includes mutations within the sequence of interest as well as within flanking sequences. They infer from this that sequences whose secondary structure is under stabilizing selection have evolved canalization of that structure [30].

We maintained that homeorhetic alleles will buffer the phenotype simultaneously against all types of perturbation. However, because of the population genetic effects of stabilizing selection, it is not true that the ability to canalize is selectively favored under the same circumstances for all classes of perturbation. This rather subtle point illustrates the distinction between the causes of variability and the fitness consequences of variability. Specifically, environmental perturbations are more likely to be the selective force promoting the fixation of homeorhetic alleles than are mutations, because there is a restricted range of strength of stabilizing selection within which the buffering of genetic variation will be favored. If stabilizing selection is too weak, homeorhetic alleles will be effectively neutral. However, if stabilizing selection is too strong, alleles that contribute to the variance of the trait will be removed from the population, leaving little or no genetic component of the phenotypic variance (Fig. 1).

This relationship has long been recognized as the negative correlation between the HERITABILITY of a trait

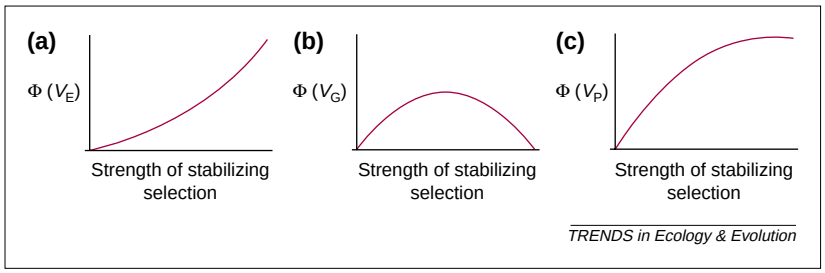

Fig. 1. Environmental variation promotes the evolution of canalization. (a) The fitness advantage associated with buffering the effects of environmental perturbations [$\Phi(V_E)$] increases as the strength of stabilizing selection on the buffered trait increases. (b) The fitness advantage associated with buffering the effects of genetic perturbations [$\Phi(V_G)$] reaches a maximum at some intermediate strength of stabilizing selection on the buffered trait. For traits under strong stabilizing selection, there will be little or no genetic component of the phenotypic variance, and thus little benefit to buffering the genetic variance. (c) The total fitness advantage associated with phenotypic buffering [$\Phi(V_P)$] is a sum of the benefits of buffering against each component of the variance. For traits under strong stabilizing selection, this benefit is due almost entirely to buffering against environmental perturbations.

and the influence of that trait on fitness [28]. As a result, the selective benefit derived from constraining traits strongly associated with fitness against the effects of mutations will be minimal. However, as canalization is the only way in which organisms can reduce the environmental component of phenotypic variation, the fitness benefit derived from constraining ENVIRONMENTAL VARIANCE increases with the strength of stabilizing selection. This qualitative result has been derived in theoretical models of the evolution of canalization [7,31], although the exact location of this selection 'window' favoring genetic canalization, and its strength relative to selection for environmental canalization depends upon the parameter values chosen for the model. The observation that the effect of the mutations:environmental variance ratio (V_m/V_e) generally lies between 10^{-3} and 10^{-5} [32] also suggests that, for a population at mutation–selection balance, the environment contributes most of the phenotypic variation of a trait, and thus produces the benefit of canalization.

These considerations assume a population that is at a mutation–selection equilibrium. For a trait undergoing a change in selection pressure, the situation is necessarily more complicated, and depends on the rates of introduction and fixation of homeorhetic alleles and removal of standing genetic variation. However, after an instantaneous increase in the strength of stabilizing selection, the waiting time for mutation to produce a homeorhetic allele should be far longer than the time it takes to remove alleles contributing variance to the trait from the population. Therefore, most homeorhetic alleles should arise in populations at mutation–selection equilibrium, and their fate will be determined by their canalizing of environmental variation.

Implications of a single mode of canalization

We have claimed here that evolved homeorhetic systems will constrain the phenotype against all manner of perturbations, and that the fitness benefit gained by buffering environmental effects will be greater than that gained by buffering mutational effects. This perspective has several consequences for other ideas about the evolution of canalization.

First, the aspects of development that are most highly canalized should be resistant to both genetic and environmental influences, in accordance with experimental results [17]. Second, selection favoring a homeorhetic allele should be a sum of the advantages provided by buffering against genetic and environmental perturbations. One could therefore refine the conclusion that there are different degrees of stabilizing selection that will promote environmental and genetic canalization [7] with a unitary fitness benefit that depends only on the overall strength of stabilizing selection. Third, the conclusion that environmental perturbations provide the major impetus for homeorhesis has implications for the plausibility of adaptively inducible canalization (Box 3).

Finally, in spite of our claim that selection for canalization should be ubiquitous, it is worth considering that homeorhetic systems can be fixed for reasons other than the action of natural selection favoring their buffering abilities. Processes that are advantageous for other reasons might be serendipitously homeorhetic. For example, the sharpness of boundaries between different cell fates specified by a morphogenic activator along its concentration gradient increases with the number of binding sites for the activator in the regulatory

Box 3. A single mode of canalization makes evolutionary capacitors implausible

Adaptively inducible canalizers ('evolutionary capacitors') are postulated to be homeorhetic mechanisms that are downregulated when organisms find themselves in stressful environments, revealing potentially adaptive genetic variation that was previously cryptic. The heat-shock protein Hsp90 has been proposed to act in just this manner in *Drosophila* [a]. Such a proposal is similar to the claim of adaptive increases in mutation rates in mutator strains of bacteria [b] but with a crucial difference. Both mechanisms will expose the organism to potentially deleterious genetic variation, either by exposing previously canalized alleles or through the generation of new mutations. However, because homeorhetic mechanisms buffer against environmental as well as genetic perturbations, downregulating them also makes the organism more sensitive to environmental influences. Unless the effects of the environment produce a more fit phenotype (a very unlikely situation), increasing the amount of environmental variance will be detrimental, especially under stressful environmental conditions. Over evolutionary time, the frequency with which a phenotypically revealed allele provides a selective advantage greater than the negative consequences of removing environmental canalization is likely to be extremely small.

The likelihood of Hsp90 acting as an evolutionary capacitor is further diminished when the biology of this protein is considered. As a heat-shock protein, Hsp90 is part of a family of genes that are inducibly upregulated under changes in temperature and other stressful conditions [c]. Unlike a homeorhetic allele of a developmental gene, there is no question that the primary function of Hsp90 is to buffer the phenotype against environmental perturbations. That Hsp90 also buffers genetic variance is clear [a], consistent with the generality of its canalization. However, if it is beneficial to constrain the phenotype against mild environmental perturbations, it must be still more beneficial to buffer against more extreme environmental perturbations. The inability of Hsp90 to buffer against a wider range of environmental conditions than it does is therefore more likely to be a coincidental feature of its mechanism of action than an adaptive trait.

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Glossary

Canalization: low variability of a genotype whereby a given phenotype remains relatively constant across different environments or genetic backgrounds.
Developmental buffering: homeorhetic mechanisms, such as feedback loops, that result in canalization.
Developmental stability: the manifestation of canalization within a single organism, such that phenotypes maintain high levels of similarity across planes of symmetry (i.e. low levels of fluctuating asymmetry).
Environmental variance: the portion of phenotypic variance in a given population that cannot be attributed to genetic factors.
Heritability: genetic variance:phenotypic variance ratio. The heritability of a trait is a measured rather than inherent property of that trait, and is a function of the genotypes and environments in which the trait is studied.
Homeorhesis: low variability of a genotype whereby a given phenotype remains relatively constant across different environments or genetic backgrounds. Homeorhesis is the developmental analog of homeostasis, and refers to the maintenance of a trajectory, rather than a state (*rheo* – Greek word meaning to flow).
Phenotypic plasticity: high variability of a genotype whereby the phenotype is relatively different across different environments, often in an adaptive manner.
Phenotypic variance: a measure of the dispersion of a phenotypic trait among individuals in a population.

regions of its target genes [33]. This 'canalization of cell type', which is very similar to Waddington's original conception of the term [1], could be the selected phenotype. However, a greater number of binding sites will also buffer the threshold width and location along the morphogenic gradient against variation in binding site affinities, such as might occur as a result of mutation or environmental input. Another example is the result that selection to increase enzyme activity will bring about a natural homeorhesis of metabolic flux (Box 2).

Analogous considerations might also be relevant with regard to the evolution of other systems of variability, such as the evolution of mutation rates [6]. Although it has been proposed that the degree of variability is itself the selected phenotype that determines the mutation rate in a given lineage [34], alternative hypotheses have not been disproved, such as the possibility that the immediate energetic costs of faithful DNA replication set the lower limit on mutation rates [6].

Ultimately, more theoretical and experimental work is required to verify these predictions definitively. One crucial question that we have not explored here is whether there is a cost to homeorhesis, either to individuals (such as a metabolic cost to maintaining homeorhetic mechanisms) or to lineages (such as a reduction in evolvability). Experimental determination of the mechanisms promoting canalization will help to address this issue. Models of the effects of stochastic processes on molecular stability [19,30] and the behavior of higher levels of biological organization, such as metabolic or developmental networks [21,22] have provided valuable insights separately; it might be that the next step is to integrate these approaches into a single model. Finally, understanding the evolution of variability will also require quantitative knowledge of the forces producing variation, such as the distribution of fitness effects of mutations, and the environmental effects on development to which organisms are exposed in nature.

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