The Opportunity for Canalization and the Evolution of Genetic Networks

Stephen R. Proulx* **and Patrick C. Phillips†**

Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, Oregon 97403-5289

Submitted June 28, 2004; Accepted October 7, 2004; Electronically published December 6, 2004

Online enhancement: appendix.

ABSTRACT: There has been a recent revival of interest in how genetic interactions evolve, spurred on by an increase in our knowledge of genetic interactions at the molecular level. Empirical work on genetic networks has revealed a surprising amount of robustness to perturbations, suggesting that robustness is an evolved feature of genetic networks. Here, we derive a general model for the evolution of canalization that can incorporate any form of perturbation. We establish an upper bound to the strength of selection on canalization that is approximately equal to the fitness load in the system. This method makes it possible to compare different forms of perturbation, including genetic, developmental, and environmental effects. In general, load that arises from mutational processes is low because the mutation rate is itself low. Mutation load can create selection for canalization in a small network that can be achieved through dominance evolution or gene duplication, and in each case selection for canalization is weak at best. In larger genetic networks, selection on genetic canalization can be reasonably strong because larger networks have higher mutational load. Because load induced through migration, segregation, developmental noise, and environmental variance is not mutation limited, each can cause strong selection for canalization.

Keywords: fitness load, genetic network, robustness, redundancy, dominance, population genetics.

One of the central themes of organismal biology is the functional integration of the organism as a whole. Changes in one part or system of an organism are likely to have effects that cascade through other systems and affect organismal functions at a variety of levels. On the one hand, these interconnections make the organism susceptible to perturbations from the environment or genetic changes, as perturbations are less likely to be localized, while on the other hand, regulatory interactions among different elements potentially allow the organism to lessen the impact of these perturbations through buffering, feedback, and compensation. When the output of a biological system is buffered against some form of perturbation, be it environmental or genetic, the system is said to be canalized (Waddington 1942).

The theme of regulatory interactions within developmental, physiological, and neurological systems has a long history within biology. More recently, interactions at the level of genetic regulation have been described in terms of genetic networks, potentially linking together thousands of elements across the genome (Furlong et al. 2001; Lee et al. 2002). These networks can be discovered by methods that reveal regulatory interactions (von Dassow et al. 2000; Ideker et al. 2001), physical interactions (Uetz et al. 2000), or biochemical/physiological interactions (Fell 1997). The ability to describe regulatory systems directly at the genetic level makes it possible to begin addressing some long-standing hypotheses regarding the functional role and long-term evolution of these systems (Waddington 1942; Schmalhausen 1949; Lerner 1954).

One of the fundamental questions regarding the evolution of genetic networks is how the structure of the network itself evolves. The most probable explanation is that network structure is determined by selection acting directly on the components (the marginal effects) of the individual network elements. Direct selection on the function of the network may also produce canalization as a by-product if networks that have stable attractors also show less sensitivity to allelic (parameter) changes (Siegal and Bergman 2002). It is possible, however, that network structure has no direct adaptive significance, with connections between genes being added and lost in a neutral, semistochastic fashion (Wagner and Fell 2001; Wagner 2003). A final possibility, and one that is the most important from a regulatory point of view, is that the network

* E-mail: proulx@proulxresearch.org.

† E-mail: pphil@uoregon.edu.

Am. Nat. 2005. Vol. 165, pp. 147–162. © 2005 by The University of Chicago. 0003-0147/2005/16502-40509$15.00. All rights reserved.

structure evolves to make the system as a whole more robust to perturbations exerted at particular nodes within the network; in other words, the system becomes canalized. This is a semantically charged area, and much effort has been spent on defining just what it means to become canalized (Wagner and Altenberg 1996; Gibson and Wagner 2000; Debat and David 2001; see table 1 for a list of definitions). However, from the most basic genetic perspective, perturbations create differences in fitness among individuals on which selection can then act. In some cases, individuals may do best by maintaining constant, robust phenotypic output of a network regardless of the perturbation (i.e., canalization), while in others it may be best to alter the output of the network (e.g., phenotypic plasticity). Thus, robustness to perturbations can be thought of as a result of canalization. Put more generally, perturbations create a kind of fitness load that produces the variation necessary for selection to operate on stabilizing the phenotype (Fisher 1958; Price 1970).

Theoretical work on the evolution of canalization has proceeded through the development of models that focus on specific genetic interactions or sources of perturbation (Gavrilets and Hastings 1994; Wagner 1996; Wagner et al. 1997; Eshel and Matessi 1998; Rice 1998; Kawecki 2000; Wagner and Mezey 2000; Hermisson et al. 2003). This approach has yet to yield a unified understanding of how canalization evolves. Our goal here is to develop a framework that can be used to study how canalization evolves in response to a variety of perturbations. Through this framework, several other phenomena can be seen to evolve via the same mechanisms that promote canalization.

In general, it is extremely difficult to determine the exact form that selection will take on an arbitrary genetic network. Indeed, even finding exact solutions involving as few as two loci can be quite difficult (Karlin and Feldman 1970; Nagylaki 1977). Instead, we take a general approach that seeks to find the upper bound on the strength of selection for robustness or canalization. If canalization is unlikely to evolve even when selection is at its strongest, then there is little point in spending time on more exact solutions. This approach also allows a wide variety of different sources of variation to be treated within the same general framework. Previous treatments of the evolution of canalization have generally taken the perturbations to the regulatory system to be external (through environmental variation) or internal (through mutation). Here, we take a general approach in which any source of variation within a population can serve to generate genetic load that can be subject to selection. To do this, we consider canalizing agents that recover some of the fitness lost by the perturbation without explicitly looking at the intermediate level of the phenotype. We show that in general the maximum strength of selection for canalization is a simple function of the fitness load generated by the perturbation regardless of the source of the perturbation (see Hermisson et al. 2002 for an alternative treatment focused on genetic load). When the fitness load is small, then the maximum strength of selection for canalization is approximately the load minus the per-gene mutation rate. This simple result allows us to consider a large set of problems under the same formalism. Thus, dominance evolution, gene duplication, and the evolution of robustness in genetic networks are all seen as canalizing effects that evolve because of the fitness load induced by mutation, segregation, migration, environmental variance, and developmental perturbations.

The Maximum Rate of Spread of a Canalizing Gene

Questions regarding the evolution of robustness or canalization can be recast as asking how novel mutations that inhibit the loss of fitness due to perturbations evolve. The source of the perturbation may be changes in the genetic background, changes in the environment, or changes in physiological status. A newly arisen canalizing gene will experience a variety of background conditions because of

Table 1: Definitions

Term	Definition
Canalization	Canalization reduces the phenotypic response to perturbations.
Canalization: genetic	A genetic element contributes to genetic canalization if it causes a reduction in phenotypic variance when expressed in a distribution of genetic backgrounds. This must be measured relative to the phenotypic variance caused by a reference genetic element expressed in the same distribution of genetic backgrounds.
Canalization: environmental	A genotype contributes to environmental canalization if it produces less phenotypic variance when exposed to a distribution of environmental states relative to a reference genotype.
Robustness	Maintenance of overall function that minimizes fitness loss in the face of perturbations.
Phenotypic plasticity	A change in phenotype for a fixed genotype in response to a change in environmental conditions.

genetic processes such as segregation and recombination and ecological processes that cause parents and offspring to experience different environmental conditions. The novel canalizing gene will spread if it is able to associate with backgrounds that allow it to have higher fitness than the rest of the population. This simple explanation highlights many of the results we discuss later; if a canalizing gene rarely experiences the backgrounds that it is beneficial in, then selection will only act weakly to spread the canalizing gene.

When discussing the evolution of canalization or the evolution of genetic interactions in general, it is important to realize that epistatic effects must be defined relative to a reference genotype (Hansen and Wagner 2001). This is particularly evident when discussing canalization because we are interested in changes in the genetic system that lead to a reduction in phenotypic variance as a response to perturbations. This could be accomplished by an allelic mutation at an existing locus, the duplication of an existing locus, the creation of a new modifier locus, or even the wholesale restructuring of the genome. For example, the modification of linkage relationships between existing alleles is a genetic change that can affect robustness without introducing a new gene in any traditional sense. Still, individuals with the new linkage arrangement have a novel genetic element (the linkage group) and can respond to natural selection for canalization. We will refer to these canalizing elements as elements, genes, or alleles to fit the context of each particular example.

We define the fitness of a novel canalizing element in background i as

$$W_i = \rho_i + (1 - \rho_i)w_i, \tag{1}$$

where $0 \leq \rho_i \leq 1$ is the degree of robustness conferred in background i and $w_i \leq 1$ is the relative fitness of background i as compared to a reference state with relative fitness 1. Again, the background state refers to the whole set of features that define an individual, including the genetic state at other loci, the environment the individual experiences, and any other relevant genomic features. The variable ρ expresses the degree of robustness that the canalizing element confers in background i. When $\rho = 1$ the deleterious effects of the background are completely masked by the canalizing element, yielding a phenotype that is identical to the reference state. As ρ declines, the masking effect of the canalizing element is reduced, and the expressed phenotype is more similar to the background phenotype.

To determine the spread of a canalizing element we need to follow changes in the background that the element is found in as well as changes in the allelic state of the canalizing gene. To simplify the situation we assume that there is unidirectional mutation from the "best" canalizing allele to other alleles and that the other alleles of the canalizing gene have reduced or equal robustness in all backgrounds. While canalizing genes must arise through mutational processes, our assumption of unidirectional mutation is justified if mutation creates functional canalizing genes much less often than mutation inactivates them. Under these assumptions the invasion of a canalizing element depends only on the spread of the best canalizing allele, so we restrict our consideration to only this best canalizing allele for the rest of the article.

Transitions between different background states may be due to the genetic features of the system or to imposed environmental or spatial variation. For instance, segregation and recombination can shift the genetic background of the canalizing gene, and the amount of linkage between all of the interacting genes will determine the probability distribution of these transitions. The environmental background can also change, but these changes will be due to less predictable mechanisms such as changes in the weather or movement between different habitats. As long as changes in the environmental background follow a Markov process, we can then define the fixed probabilities that cause a novel canalizing element to move between backgrounds.

To calculate the opportunity for canalization we need to determine the spread of a novel canalizing element. The analysis is based on assumptions that are commonly used in several approaches to evolutionary theory, including genetic invasion analysis (Feldman and Karlin 1971), evolutionarily stable strategy (ESS) analysis (Shaw and Mohler 1953; Maynard Smith 1982), and adaptive dynamics (Metz et al. 1992; Dieckmann and Law 1996). We assume that before the novel genetic element appears, the resident population achieves a stable stationary distribution of states, which will in turn produce the genetic and environmental background. For particular examples that we discuss, the resident dynamics are explicitly defined and the stable states determined. We then follow the spread of a rare novel element that must interact with the set of backgrounds produced by the resident dynamics. We do this by assuming that the frequency of the class of individuals carrying the novel element is small, on the order of a term ϵ. While this condition of rarity holds, interactions between individuals carrying the novel element will typically be on the order of ϵ^2, which we ignore. Because of these assumptions, the spread of the novel element can be expressed using a linear matrix equation that depends on the steady state of the resident population.

We assume that a single, well-mixed population consists of individuals that can be indexed by their background state, i. When a novel genetic element appears in this population, either as an allelic variant at a preexisting locus

or as a new gene, the number of individuals carrying the novel allele can be described by the recursion

$$v_i(t+1) = \frac{1-\mu_c}{\bar{w}} \sum_j v_j(t) W_j T_{ji}, \tag{2}$$

where $v_i(t)$ is the number of individuals in background i carrying the novel allele at time t, μ_c is the deleterious mutation rate of the novel allele, $\bar{w}$ is the mean fitness in the population before the novel element is introduced, T_{ji} is the probability that an individual offspring produced by a background j individual will end up in background i as an adult, and W_j is the fitness of individuals carrying the novel allele in background j. For the rest of the article we will suppress the t. Note that to reduce the number of subscripts we have defined the fitness of individuals in background i without the novel allele as w_i while the fitness of individuals with the novel allele is denoted as W_i. The matrix **T** represents the probability that a novel allele from a state i individual will end up in a state j individual following reproduction. This matrix does not take selection into account but includes effects from the mating system, segregation, recombination, and environmental effects. The **T** matrix also is affected by the equilibrium frequencies of resident individuals with each background.

We can convert equation (2) into the matrix equation $\mathbf{v}(t+1) = \mathbf{v}(t)\mathbf{R}$ by defining the matrix

$$\mathbf{R} = \frac{1-\mu_c}{\bar{w}} \mathbf{FT}, \tag{3}$$

where **F** is a matrix with the fitness values W_j along the diagonal and 0 elsewhere. Using the eigenvalue equation $\lambda \mathbf{v}^* = \mathbf{v}^* \mathbf{R}$, we can sum over the states to get

$$\lambda \sum_i v_i^* = \frac{1-\mu_c}{\bar{w}} \sum_i \sum_j v_j^* W_j T_{ji}. \tag{4}$$

The summations on the right-hand side can be rearranged and simplified by noting that $\sum_i T_{ji} = 1$ for every j. This is because the **T** matrix represents the distribution of offspring among the possible states, and each offspring must end up in some state. Defining $q_j = v_j^*/(\sum_i v_i^*)$ and $\widehat{W} = \sum_j q_j W_j$ gives

$$\lambda = \frac{1-\mu_c}{\bar{w}} \widehat{W}. \tag{5}$$

The term $\widehat{W}$ represents the realized average fitness of the novel element, given the distribution it achieves while it is spreading (i.e., along the eigenvector). Thus, equation (5) shows that the average fitness of the canalizing element determines whether the novel element will spread. This average fitness value will typically be weighted toward states that are more common in the original population as well as states that have higher fitness with the novel allele. This means that canalization of rare states will contribute less to selection on canalization than canalization of common states.

While it can be quite difficult to solve for the spread of the canalizing gene in general (because the distribution of background states is usually unknown), we can find it for some special cases and also find the upper bound on selection for canalization. When the level of robustness is the same for all states (i.e., $\rho_i = \rho$), then equation (5) simplifies to

$$\lambda = \frac{1-\mu_c}{\bar{w}} [\rho + (1-\rho)\hat{w}], \tag{6}$$

where $\hat{w} = \sum_j q_j w_j$. The best-case scenario for the spread of a canalizing gene occurs when the gene confers perfect robustness and $\rho_i = 1$, so that an upper bound on the strength of selection for canalization, defined as $s_c = \lambda - 1$, is given by

$$s_c \le \frac{1-\mu_c}{\bar{w}} - 1. \tag{7}$$

This result is quite intuitive; selection goes up as $\bar{w}$ goes down because there is more fitness load for a canalizing gene to repair. However, mutation at the canalizing locus itself imposes a direct cost. This mutation cost is typically left out of ESS analyses because it is usually thought to be on a smaller order of magnitude than selection on typical adaptations. In this analysis, as we will show, it cannot be ignored because it is often on the same order of magnitude as selection for canalization. Note that the selection coefficient, s, is typically written with only positive terms, but we have included the force of mutation in s_c.

It is informative to express this in terms of the fitness load in the system. There have been many different formalizations of the genetic load, but here we refer to the fitness load as an umbrella term for the reduction in fitness due to any sort of perturbation. The standard definition of genetic load is

$$L = \frac{w - \bar{w}}{w}, \tag{8}$$

where w is the fitness of a "reference type" and $\bar{w}$ is the mean fitness in the population (Crow 1958). Traditionally, the reference type is taken to be the genotype with the highest fitness, or the genotype with no mutations. Here, we extend this notion by defining the fitness load with

respect to the reference background with the highest absolute fitness and set the reference relative fitness to 1. In this view, the fitness load could be due to mutation, to segregation, to recombination, to migration, or entirely to environmental fluctuations. When both the load and the mutation rate are small and of similar magnitudes, then

$$s_c \lessapprox L - \mu_c. \tag{9}$$

This provides a simple upper bound on the strength of selection for canalization as the load in the system minus the mutation rate of the canalization gene. Thus, if the load is on the order of the per-gene mutation rate, then there is no selection for canalization at all. Under all circumstances, selection for canalization cannot be greater than the load in the system.

Equation (9) establishes an upper bound to the strength of selection on a canalizing element. This makes some intuitive sense because a purely canalizing element can only be subject to selection when it "fixes" something that is "broken," as reflected in the load of the system in the absence of the canalizing element. This result immediately allows us to compare the load produced by different sources of variance to understand why the strength of selection on canalization differs in those systems. This is only a gross measure because the realized strength of selection on canalization will also depend on the amount of robustness that is feasible (limitations on ρ_i) and the way that the canalizing element is shuffled among backgrounds (realizations of q_i). The feasibility of canalization is dependent on the biological details of the system; there are certainly limits to the ability of single genetic changes to mask variability. The changing associations of the background will be determined by the preexisting genetic structure of the organism and the structure of environmental changes. These effects can limit selection on the canalizing element if they prevent it from becoming associated with the backgrounds that it canalizes best.

Genetic load has played an important role in population genetics and has been extensively studied and reviewed (Crow 1970). In many cases, we can make use of this extensive literature to determine the upper bound on selection for canalization. In other cases we must develop estimates of the maximum fitness load in order to establish an upper bound selection for canalization.

The Opportunity for Canalization in Gene Networks

In the previous section we showed that the maximum strength of selection for canalization is a simple function of the fitness load irrespective of the source of the variance. All genes are subject to mutation, and mutational load has played an important part in many facets of evolutionary thinking (Haldane 1937; Kondrashov 1984; Crow 1993; Cherry 2002). The amount of load that mutation generates is known to depend on many factors, including the number of genes, the mutation rate, the form of epistasis, the mating system, and population structure (Haldane 1937; Crow 1970, 1993; Kondrashov 1984; Charlesworth et al. 1990; Burger 2000; Whitlock 2002). In this section we consider the amount of load that is generated by genetic networks of different complexities and consider how novel genetic elements that contribute to genetic robustness might spread.

We define a genetic network as a group of loci that interact to produce a phenotype that relates to fitness. These loci can interact in any number of ways, such as through transcription regulation, by producing proteins that interact, or by producing morphological traits that exhibit fitness trade-offs. Robustness can evolve through the allelic modification of a locus or through the addition of a novel locus that mediates the interactions. Novel networks can even be created from groups of noninteracting loci when a gene that interacts with preexisting genes arises. In this case, the canalizing element actually creates the network.

In order to understand the opportunity for canalization of the mutational load in genetic networks, we need to know how the topology of genetic networks generates the genetic load. Rather than focusing on the mechanistic dynamics of genetic networks and the resulting epistatic interactions, we focus directly on the genetic load that can be generated by such networks. By determining the circumstances that make canalization a feasible explanation for genetic network evolution, we can focus our modeling efforts on problems that are likely to play a role in the evolution of real biological systems. To do this, we consider the epistatic relationships that provide the least and greatest amounts of load.

Evolution of Two-Gene Networks through Canalization

The simplest genetic network consists of a single haploid gene. In this scenario there is no epistasis or dominance to complicate the issue, so canalization can only evolve through the addition of a novel genetic element that masks the effect of mutations at the primary locus. One possible mechanism for canalization in this system is the duplication of the original gene. If a single wild-type copy of the gene is sufficient to produce the wild-type fitness, then the gene duplicate is said to be redundant, and the novel network exhibits epistasis. This two-gene network exhibits epistasis and shows a reduction in the variability of fitness to perturbation at either locus and is thus canalized (Wagner and Altenberg 1996).

Mutation-selection balance at a single haploid locus is

well known to generate a mutational load of μ, the rate of mutation from the wild-type allele to a deleterious state (Crow 1993; Burger 2000). This result is robust to changes in the number of deleterious alleles and the fitness map (Burger 2000). Direct application of equation (9) shows that

$$s_c \leq \mu - \mu_d, \tag{10}$$

where μ_d is the mutation rate at the duplicated locus. In the case of a gene duplication, it is unlikely that the mutation rate will go down, and the most likely situation is that $\mu = \mu_d$. Thus, redundancy can only evolve through selection if it is accompanied by a reduction in the mutation rate. In that case, we could see the performance of a function shift from one gene to another with a lower mutation rate, but even in this scenario long-term stability of the canalized system is unlikely, as the gene with the higher mutation rate will eventually go extinct (Nowak et al. 1997; Phillips and Johnson 1998).

This simple analysis of the transition from a one-gene into a two-gene network highlights the forces that operate on network evolution in general. Any factor that increases the size of the mutational target experiences a direct cost and thus must be balanced by an increase in the mean fitness of individuals carrying the novel genetic element if it is to spread.

Canalization through Dominance Evolution

The mutational load generated by a single haploid gene is not large enough to create selection for canalization through gene duplication because the direct cost of mutation is on the same order as the benefit of reducing the load. However, a single diploid locus can create a mutational load of up to twice the mutation rate (Crow and Kimura 1970). This provides an opportunity for selection on a gene that can modify the interaction between alleles at the original locus, leading to the evolution of dominance of the wild-type allele (Fisher 1928). This system was extensively studied throughout the twentieth century, with the general conclusion that direct selection for dominance modification is weak and other forces will play a larger role (given that the population is at mutation-selection balance; Fisher 1928; Wright 1929, 1934; Haldane 1930; Waddington 1942; Feldman and Karlin 1971; Keightley 1996; Bourguet 1999; Hurst and Randerson 2000; Omholt et al. 2000; Otto and Yong 2002). Dominance evolution can be viewed as canalization by noting that the gene that confers dominance at the original locus reduces the change in fitness in response to genetic variance.

We can use our framework to show that the maximum strength of selection on dominance via genetic canalization is limited by the mutation rate in equilibrium populations. Depending on the degree of dominance, the mutation load at a diploid locus can range from μ to 2μ (where μ is the per-allele mutation rate). This gives the maximum opportunity for canalization through dominance modification as

$$s_c \lessapprox 2\mu - \mu_d, \tag{11}$$

where μ is the mutation rate at the major locus and μ_d is the mutation rate at the locus controlling dominance (this is equivalent to the result found by Wright 1934; see the appendix in the online edition of the *American Naturalist* for a full derivation). This means that when mutation acts equally on the major locus and the modifier, then the strength of selection is about half of the load.

A single diploid locus is, in effect, a genetic network with two sites. When the potential canalizing element can only effect the load generated by a single locus, then the direct cost of mutation is expected to be similar to the maximum benefit of reducing the load. A single diploid locus can generate more load, and therefore selection for canalization can be larger than the direct cost of mutation on the novel gene, but even in the best-case scenario it is only expected to be equal to the per-gene mutation rate.

Canalization of the Mutation Load in Larger Genetic Networks

So far we have examined canalization of the mutational load generated by a single locus and canalized either through the creation of a genetic network or through modification of the existing dominance interactions. The mutation load generated by systems with only a few genes cannot be very large, and so the strength of selection on canalization is small or nonexistent. Larger networks can produce larger load because of the larger total number of mutational targets (de Visser et al. 2003) and because of epistatic interactions among loci. In order to understand how the total strength of canalizing selection depends on genetic network structure, we consider three extremes of network structure: nonepistatic networks, redundant networks, and fragile networks (fig. 1).

Mutation Load in Nonepistatic Networks. Consider a haploid genetic network that contains n genes that do not have epistatic interactions. If the mutation rates at all loci are the same, then load is given by $L = 1 - (1 - \mu)^n$ under very general conditions (Crow 1993; Burger 2000). The maximum strength of selection on a canalizing element will thus be

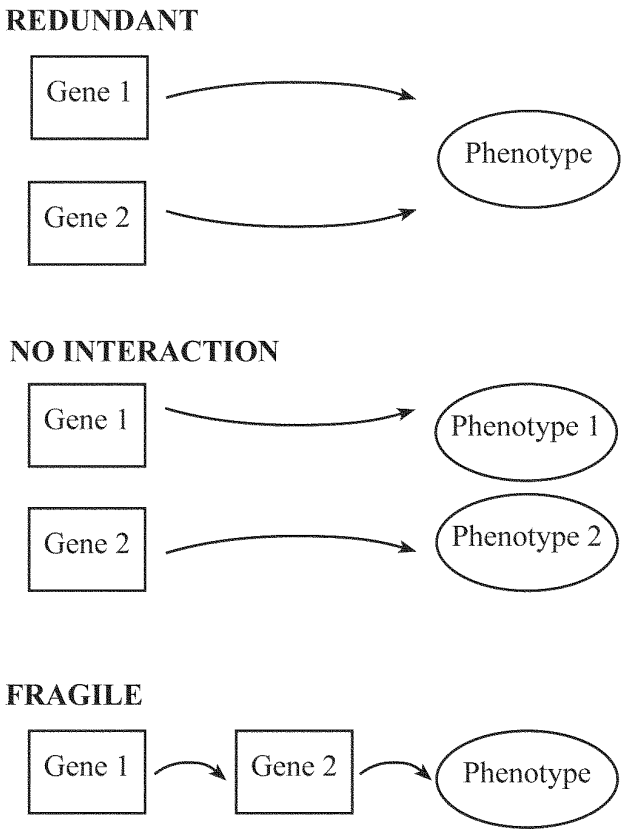

Figure 1: Possible structures of two-locus genetic networks. In a redundant network, a product is independently produced by two separate loci. As long as one gene functions, the product is produced, and no loss of fitness occurs. In a nonepistatic network, each gene produces a separate product. Fitness is reduced by a selection factor for each gene that does not function. In a fragile pathway, the product of one gene is acted on by the other gene. If either gene fails to function, no product will be produced, and a fitness cost is incurred.

$$s_c \leq \frac{1-\mu_c}{(1-\mu)^n} - 1. \tag{12}$$

We can use some approximations to develop a sense for how the strength of selection depends on μ and n. We can approximate equation (12) under the assumption that both μ and μ_c are small and of the same magnitude to get

$$s_c \lessapprox n\mu - \mu_c. \tag{13}$$

Just as in the single-gene duplication case, there is a direct cost of mutation that the canalizing element must overcome, represented by the term involving μ_c. Selection for canalization increases with n just as the mutation load does. Equation (13) indicates that selection for genetic canalization will only be on the order of the mutation rate but will increase with the number of genes in the network.

To achieve a selection coefficient of 10^{-3}, approximately 100 genes are required when the mutation rate is 10^{-5}, while only 10 genes are required to generate a selection coefficient of 10^{-4} (fig. 2). Thus, for nonepistatically interacting genetic networks and reasonable values for the per-gene mutation rate, genetic robustness is only likely to evolve when either the network affected is large or population size is large.

Mutation Load in Redundant Networks. An extreme case of negative epistasis is exemplified by synthetic lethal, or synthetic deleterious, phenotypes (Phillips and Johnson 1998). A synthetic lethal phenotype is said to occur when mutant alleles at several loci are required to produce a lethal phenotype but each mutant allele in a wild-type background has the wild-type fitness. In our genetic network context, this means that the network performs its function perfectly if there is a wild-type allele at one or more loci but fails to function if no wild-type alleles are present. We assume that if the network under consideration fails to operate, then organismal fitness is reduced by s. We show in the appendix ("Mutation Load in Genetic Networks") that the maximum load for this system is

$$L = \mu_{\min}, \tag{14}$$

where $\mu_{\min}$ is the minimum mutation rate at any locus in the network. Note that load does not depend on the cost per mutation, s. This represents a lower bound to the mutational load of a network where the load has basically been collapsed onto a single locus, which is able to keep

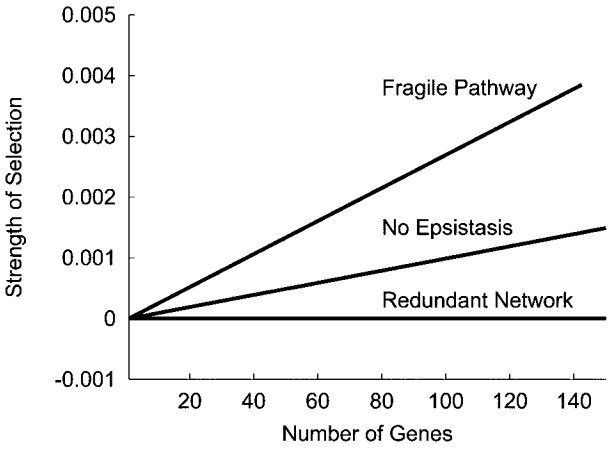

Figure 2: Selection for the case of no epistasis calculated from equation (12), for the redundant network from equation (15), and for the fragile pathway from equation (17). The mutation rate is set at 10^{-5}. The fragile pathway generates the most load and so produces the greatest strength of selection on canalization.

the network functioning regardless of the mutations that have accumulated at the other loci. The maximum strength of selection to first order in the mutation rate is given by

$$s_c \lessapprox \mu_{\min} - \mu_c. \tag{15}$$

Thus, a novel canalizing mutation can only spread if it can further reduce the mutational target. This is akin to the evolution of reduced mutation rates, although through a mechanism that operates on a more local genetic level (Johnson 1999; Sniegowski et al. 2000). In essence, such a redundant system is already so canalized that there is essentially no selection for further canalization.

Mutation Load in Fragile Linear Pathways. Positive epistasis represents the best-case scenario for the evolution of robustness because it generates the most genetic load (Phillips et al. 2000). Positive epistasis will occur when the reduction in fitness of individuals with multiple mutations is less than would be expected based on a multiplicative fitness model. This could naturally arise in gene networks that are simple linear pathways (fig. 1), where any single knockout disrupts the network. The most extreme case is when the network fails to function if the wild-type allele is missing at any locus, causing a loss in total fitness of magnitude *s*. In the appendix ("Mutation Load in Genetic Networks"), we show that when the mutation rates are all equal, then the maximum load possible in fragile networks is

$$L \le n\mu \frac{n^{n-1}}{(n-1)^{n-1}}, \tag{16}$$

where n is the number of genes in the network. This expression for L can be substituted into equation (9) to give an upper bound on the strength of selection for canalization:

$$s_c \le n\mu \frac{n^{n-1}}{(n-1)^{n-1}} - \mu_c. \tag{17}$$

The fraction in equation (17) increases with n and has a limiting value of e, the base of the natural logarithm. When the number of genes in the network is large, the maximum strength of selection can be approximated by

$$s_c \le n\mu e - \mu_c. \tag{18}$$

Thus, a fragile network can have up to a factor e more opportunity for canalization than a nonepistatic network.

Because the equilibrium load depends on the selective cost in this model and because equation (16) only estimates the maximum load, we performed a numerical investigation of load. Figure 3 shows that load is only increased over the nonepistatic model for a small range of parameters. This suggests that in practice the load in fragile pathways is probably not much more than in nonepistatic networks. Given that this is likely to be the most extreme form of genetic network from a load perspective, examination of more specialized network topologies does not affect our general conclusions about the strength of selection for canalization of the mutation load.

Other Forms of Perturbation

The magnitude of the mutational load in genetic networks is limited by the size of the network, by the existing ep-

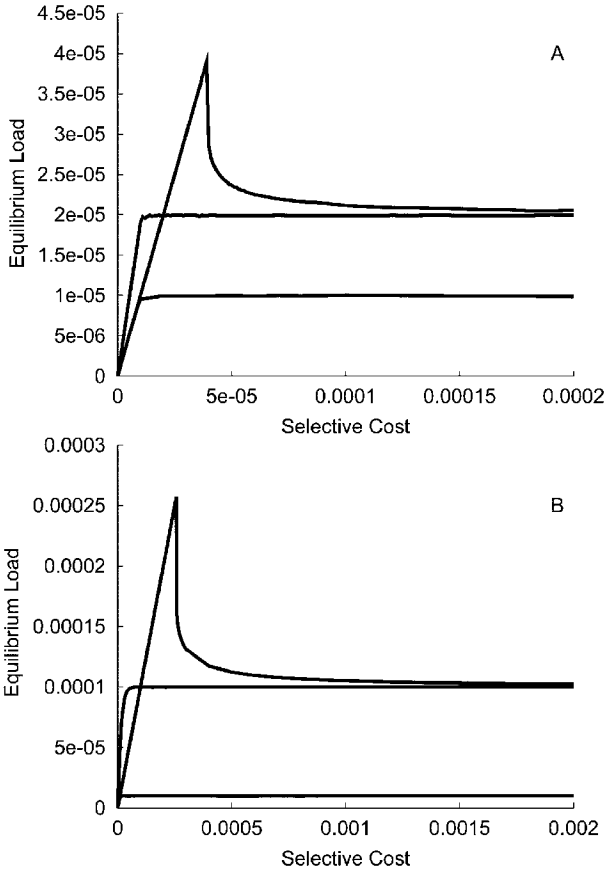

Figure 3: Numerical calculation of the equilibrium genetic load in a genetic network. For all curves, the mutation rate was set to 10^{-5}. The top peaked curve is for fragile pathways, the middle curve is for nonepistatic networks, and the bottom curve is for completely redundant networks. *A* shows the equilibrium load for a two-gene network, while *B* shows the load for a 10-gene network. Selection for canalization is always low in the redundant network. The fragile and nonepistatic networks have similar levels of load except for a small range of the selection coefficient near the error threshold.

istatic interactions, and most importantly by the magnitude of the deleterious mutation rate. Several other forms of perturbation, however, are known to generate large fitness loads even when only a small number of genes are involved (see Crow 1993 for a review). These perturbations can be caused by genetic phenomena, such as segregation or migration, or by environmental perturbations on any number of temporal or spatial scales. We can directly apply our general results to these scenarios based on the load that develops due to each alternative source of variation. Table 2 summarizes our results for the opportunity for canalization under each type of perturbation.

Segregation Load. The genetic load due to segregation can be much larger than load due to mutation accumulation because maladapted individuals are produced at large frequencies (Muller 1950; Dobzhansky 1955; Crow 1993). For a single locus with two alleles, where the heterozygote has the greatest fitness, the load is $\tilde{s}/2$ and

$$s_c \leq \frac{\tilde{s}}{2} - \mu_c, \tag{19}$$

where $\tilde{s}$ is the harmonic mean of the selection coefficients against homozygotes (Crow and Kimura 1970). In the most extreme case where homozygotes have zero fitness, the load can be as high as one-half.

One way to canalize the segregation load is through gene duplication (Hammerstein 1996; Otto and Yong 2002). Duplication allows each locus to become fixed for a single allele and allows the stable inheritance of a genotype containing both alleles. Under the assumptions that fitness depends on having at least one copy of each allele and that the fitness costs paid by both homozygotes are small and equal to s, the opportunity for canalization is

$$s_c \leq \frac{s}{4} - \mu_c. \tag{20}$$

(Otto and Yong 2002; see appendix, "Heterozygote Advantage"). Because the segregation load can be large, selection for canalization via gene duplication can easily overcome the mutational loss of new duplicates and play a role in the creation of genetic networks that remove heterozygote advantage.

Migration Load. Movement of genotypes among environments that have different selection regimes is similar to mutation in that genetic variants are constantly introduced. Migration differs from mutation because the rate of influx can be large, immigrant genotypes may be predictable, and entire haplotypes are exchanged. Selection to reduce the deleterious effects of immigrant alleles can be seen as a form of canalization because the effect of genetic variation at a spatially polymorphic locus is reduced (Mayr 1960; Bourguet 1999; Otto and Bourguet 1999).

In the classic mainland-island scenario (Wright 1931), migration load is generated by input of nonadapted alleles through migration. The load is highest when the maladapted immigrant allele is dominant, giving a load of approximately twice the migration rate (m) under weak migration. When the homogenizing effect of migration at the modifier locus is taken into account (see appendix, "Migration Load"), the opportunity for canalization becomes

$$s_c \leq m - \mu_c. \tag{21}$$

The strength of selection on this dominance modifier is reduced to less than half of the load because of the assumption that immigrants never carry the modifier allele. Because migration rates can be much higher than mutation rates, the migration load can generate strong selection for canalization.

The genetic perturbation caused by mutation generates load that can be canalized by creating a genetic network. Migration pressure causes the evolution of genetic cana-

Table 2: Major types of canalization

Type of perturbation	Maximum value of s_c	Effect
Single gene	0	No selection because mutation cost is equal to potential benefit.
Dominance	μ	Weak selection. Similar to a two-locus network.
Mutation in networks	$\mu e(n-1)$	Generally weak but increases with the number of interacting genes.
Segregation load	$s/4$	Strong, can generate large quantities of load.
Individual variation	L	Variable. Selection maximizes mean fitness. Load will be large when the frequency and fitness effects of disturbance are high.
Seasonal variation	L	Variable. Selection maximizes geometric mean of fitness and reduces variance in fitness, but may select for phenotypic variance.
Migration load	m	Strong, increasing with the migration rate.

Note: s_c is the maximum strength of selection for canalization.

lization, which itself would be difficult to distinguish from canalization of the mutation load. However, because the influx of genetic variants caused by migration is more predictable than the genetic perturbations due to mutation, networks that canalize the migration load may be more feasible than networks that canalize the mutation load (fig. 4).

Individual Environmental Variance. When the environment varies on a small spatial scale, we expect individuals to experience different environmental conditions than other members of their population. This could occur because the external microhabitat that individuals inhabit differs or because of differences in the internal "environment" during embryogenesis, which may not be easily attributable to any measurable feature of the external environment. This developmental noise may differ functionally from fine-scale spatial variance but produces the same type of selection (Frank and Slatkin 1990; Yoshimura and Clark 1991). In the adaptationist literature, this form of variance is thought of as selecting for the increase in mean fitness an individual would obtain after averaging over the possible environmental states (as long as population size is reasonably large; Gillespie 1974; Bulmer 1984; Yoshimura and Clark 1991; Proulx 2000).

Previous work on canalization of environmental variation has focused on the genetic features of canalization and pleiotropic interactions between canalization and environmental adaptation (Gavrilets and Hastings 1994; Wagner et al. 1997). Because this form of selection generally leads to the presence of a single strategy (Seger and Brockmann 1987; Ellner 1996), we assume that a single genotype at the environmental adaptation locus is present at equilibrium and therefore that the fitness load is due entirely to environmental variation. The opportunity for canalization is thus given by

$$s_c \leq \sum_e s(e)p(e), \tag{22}$$

where e represents the environmental variable, $s(e)$ is the fitness decrement paid when raised in environment e, and $p(e)$ is the frequency of environment e. In this context, the reference background is defined by the environment (or more generally, an environment and a genotype) that produces the largest fitness. Canalization can act by increasing fitness in some environments while leaving fitness in the reference environment unchanged. To utilize our matrix approach, we define the transition between classes based on the process describing the environmental variation (as long as the environmental pattern can be represented by a Markov process).

In this context, a reduction in $s(e)$ will increase mean fitness and can be considered canalization, at least at the level of organismal fitness. In the context of developmental noise, the mean of s could be reduced by classical canalization; that is, a genetic change that allows the phenotype to remain closer to the optimum would reduce s and increase mean fitness. The amount of load produced by individual environmental variance can be large because it is not limited by the mutation rate. The fitness load can be high either because the rate of environmental disturbance is large or because the fitness effects of disturbance are large (Pál and Hurst 2000).

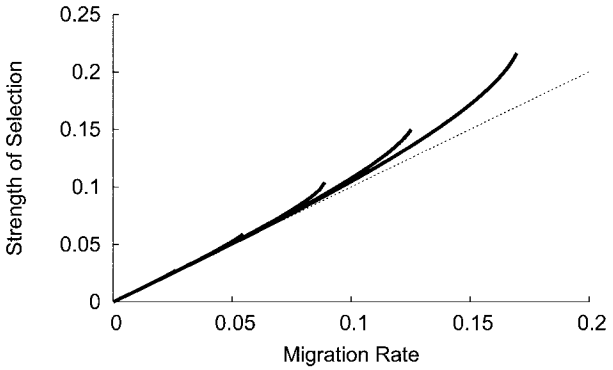

Figure 4: Selection for canalization under migration-selection balance. The parameter values for the curves are $s = 0.1, 0.2, 0.3, 0.4$, and 0.5. The actual values of s_c are plotted along with the small m approximation (*dotted line*).

The major limitation to the evolution of environmental canalization is really the feasibility of achieving canalization without compromising other systems (Wagner et al. 1997). The opportunity for canalization in this case does not depend directly on the number of genes that contribute to phenotype, but the genetic network structure may itself determine the feasibility of canalization.

Population Level Environmental Variance. In addition to the unique environment experienced by each individual, populations experience environmental fluctuations in both space and time. When all individuals in the population face the same environment but environmental fluctuations affect each generation differently, then selection acts on the entire distribution of fitness effects (Dempster 1955; Levins 1962; Gillespie 1974; Tuljapurkar 1990). For infinite populations without overlapping generations, selection acts to maximize the geometric mean of fitness (Caswell 2001; but see Proulx and Day 2001 for a discussion of the geometric mean criterion in finite populations). The geometric mean of fitness is increased by reducing the variance in fitness, and this again leads to canalization when re-

duced phenotypic variance results in reduced variance in fitness.

As in the case of developmental noise, only a single genotype is expected to persist, so long as generations are not overlapping and there is no overdominance of the geometric mean (Chesson and Warner 1981; Ellner 1984; Chesson and Ellner 1989). The load can be calculated using an equation similar to equation (22) but using the geometric mean of fitness. The opportunity for canalization is

$$s_c \leq 1 - e^{\{\Sigma_e \log[1-s(e)]p(e)\}}. \quad (23)$$

Because the geometric mean of a random variable is always lower than its arithmetic mean, the load due to seasonal variance will be larger than the load due to developmental noise for the same underlying pattern of variation. This causes selection against variance per se. In this case, canalization could occur even at the expense of mean fitness. For example, if there is a trade-off between genotypes that produce stable phenotypes in the face of environmental variance and genotypes that produce higher fitness phenotypes in the most common environment, then the stable genotype could have a higher geometric mean fitness.

An important caveat here is that seasonal variation can select for variable developmental strategies even as it selects for reduced variance in fitness and reduces genetic variation (Levins 1962; Gillespie 1973; Seger and Brockmann 1987). The evolution of canalization in response to seasonal variance will depend on whether a single developmental strategy produces the highest geometric mean fitness or whether a so-called adaptive coin-flipping strategy produces the highest geometric mean fitness (Kaplan and Cooper 1984). In this case, even though variance in fitness is selected against, increased phenotypic variance (albeit nongenetic) can be selected for.

These considerations suggest that a single genotype that limits the effect of seasonal variance can spread both because of an increase in mean fitness and a decrease in fitness variance. Kawecki (2000) showed that in some situations, genetic canalization can limit the short-term evolutionary responses to selection and allow a single genotype with a high geometric mean fitness to persist. As in the case of environmental variance at the individual level, the number of genes contributing to the phenotype do not determine the load directly. However, the evolution of buffering mechanisms that depend on integrating environmental information and using feedback probably require the creation of genetic networks.

Discussion

Canalization is a difficult process to model because it explicitly depends on variance. Many theoretical treatments of evolutionary processes minimize the role of variance either by ignoring it entirely or by assuming that it is small and regular (e.g., Lande 1976; Yoshimura and Clark 1991; Dieckmann and Law 1996; Taylor and Day 1997). This has lead workers to develop specific, explicitly genetical, dynamic models of canalization to understand how canalization evolves in a variety of contexts (Clark 1994; Gavrilets and Hastings 1994; Nowak et al. 1997; Wagner et al. 1997; Eshel and Matessi 1998; Wagner 1999; Kawecki 2000; Otto and Yong 2002; de Visser et al. 2003; Hermisson et al. 2003). We have taken an alternative approach and derived a simple expression for the maximum strength of selection on a rare canalizing element. This approach applies to a range of scenarios and allows us to compare genetic and environmental canalization on the same footing.

Because canalization is the stabilization of the phenotype in the face of a perturbation, it can at most return fitness to that of an individual in the "best" state. We can approximate the maximum selection for canalization as simply $L - \mu$ (load minus mutation rate). This is because the fitness benefit of the canalizing modifier is at most equal to the fitness load in the population and because all genes must pay a direct cost of mutation. This direct cost of mutation is left out of most evolutionary models because it is generally assumed to be much smaller than the selection coefficient. This assumption is not valid, however, when mutation is the main force driving evolution (Johnson 1999).

The amount of load generated by any perturbation depends on the amount of canalization that has already occurred. For instance, even if environmental conditions are constantly in flux, the current strength of selection will depend on the fitness cost associated with the environmental variance. Similarly, canalization of the mutation load can reduce the magnitude of the fitness load by modifying the epistatic interactions between existing allelic variants. However, once a canalizing modifier has gone to fixation, novel mutations may appear that are not yet canalized. This could lead to a constant cycle of the buildup of load followed by the canalization of that load.

The approach we have taken contrasts with recent models of canalization in that we model only the invasion of a rare canalizing gene. This allows us to create more general models and compare the strength of selection on canalization through different modes of selection. The drawback of this approach is that it does not guarantee that a canalizing gene with a large selection coefficient when rare will spread to fixation. However, our main goal is to identify limits to selection on canalization, and selection is likely to be strongest when the canalizing allele is rare.

We model the spread of new genetic elements when they are rare because any new gene must survive this pe-

riod if it is to persist. While nonlinear dynamics may affect the eventual fixation of a canalizing element, these effects are only important if canalizing genes are not lost when rare. Because our analysis reveals that selection for canalization of genetic networks is usually weak, it is not clear that it is worth further effort modeling the evolution of canalization in particular genetic networks. Future effort would be better spent on determining the direct selective advantage that different network structures provide, and this work will require explicit modeling of genetic network dynamics.

The Evolution of Genetic Networks

Genetic networks are likely to be involved in canalization of both genetic and environmental perturbations. For genetic canalization, genetic networks are involved almost by definition. Although a form of genetic canalization can act through only a single locus (or single linkage unit; Eigen and Schuster 1977; Nowak 1992; van Nimwegen et al. 1999; Wilke et al. 2001), genetic canalization is only likely to evolve when many genes interact epistatically (Wagner et al. 1997; Hermisson et al. 2003). Genetic canalization involves modification of the epistatic interactions between genes, which can occur either through allelic changes at existing loci or through the incorporation of new genes into the network. For example, in the classic example of the evolution of dominance, Fisher (1928) invoked a modifier of the dominance interaction at the major locus. The addition of a dominance modifier creates a two-gene network that epistatically determines the trait value. Likewise, the evolution of redundancy and reduction of the segregation load can occur through gene duplication, again producing a two-gene network (Clark 1994; Nowak et al. 1997; Otto and Bourguet 1999; Wagner 1999; Otto and Yong 2002).

While gene duplication can bring about canalization through redundancy, regulatory mechanisms can canalize both genetic and environmental perturbations. For instance, if genetic or environmental perturbations alter the rate at which an enzyme catalyzes a reaction or the binding efficiency of a signaling protein, then regulation through feedback can maintain global properties of the system. Examples of this include the chemotaxis network of bacteria, the segment polarity network, and metabolic systems regulated by the substrate, such as the lac operon and galactose metabolism (Barkai and Leibler 1997; von Dassow et al. 2000; Ideker et al. 2001). Canalization via regulation is implicit in Waddington's (1942) description of canalization but has to some degree been neglected in recent attempts to explain the congruence of environmental and genetic canalization based on enzyme kinetics and structural stability (Ancel and Fontana 2000; Hurst and Randerson 2000; Meiklejohn and Hartl 2002; de Visser et al. 2003). Canalization through feedback regulation can only work on perturbations that affect quantitative components of networks (as opposed to structural components) and is likely to canalize both genetic and environmental perturbations.

Canalization of the mutation load can occur through dominance modification, gene duplication, modularization, or modification of genetic interactions. Duplication of a single haploid locus cannot be explained by selection for canalization of the mutation load, however, because the direct cost of mutation on the duplicated locus will outweigh the benefit of even complete canalization. Nevertheless, dominance modification at a single locus can be selected for because a single diploid locus is equivalent to a two-gene network and can generate load that is greater than the mutation rate. However, the strength of selection on canalization of the mutation load through dominance modification is only on the order of the mutation rate. Even placed in the context of larger genetic networks, dominance modification and gene duplication will not be associated with large selection coefficients if the locus in question interacts nonepistatically with other members of the network. Although other network structures will accumulate load differently, the opportunity for canalization will necessarily be lower than the upper bound described here. Unless a new gene buffers mutations at a large number of loci, selection for canalization of the mutation load will be negligibly weak.

Extending these results to specific genetic networks is simultaneously trivial and difficult. Our main results deal with the genetic networks that realize the least and greatest mutational loads for a given number of genes. Other genetic networks will fall in between the extremes of completely fragile and completely redundant, so they have less opportunity for canalization than fragile networks. To illustrate, we briefly consider the effects of epistatic modularity in a genetic network. We call two modules in a network epistatically modular if total fitness is a product of module specific fitness. The load in epistatically modular networks is approximately the sum of the load in each module (see appendix, "Epistatically Modular Networks"). This makes it easy to show that in epistatically modular networks consisting of n loci in two components of equal size, the opportunity for canalization is bounded by

$$s_c \leq \mu n \frac{(n/2)^{n/2-1}}{(n/2-1)^{n/2-1}}, \tag{24}$$

which is less than s_c for a fragile network with a total of n genes. However, there is no guarantee that modularization will reduce the load. For instance, if the original

network is already redundant, then modularization can actually increase the load.

If selection for gene duplication and dominance modification are unlikely to explain the addition of genes to networks, is there a role for canalization of the mutation load in the creation of genetic networks? It seems that high selection coefficients can only be generated when the canalizing gene interacts directly with a large number of preexisting genes. This requires a single evolutionary step that reduces load due to many genes. The event could be the modification of an existing gene that already interacts with many partners or a genome-wide event such as polyploidization. For example, Otto and Whitton (2000) have shown that polyploidization can temporarily reduce the mutation load as a form of canalization.

One group of proteins that interact with many other partners is the heat shock proteins (Hsp). Heat shock proteins aid in protein folding for a large group of proteins that do not necessarily interact with each other (Rutherford and Lindquist 1998; Bergman and Siegal 2003). Figure 2 shows that if an Hsp interacts with approximately 100 genes that have mutation rates of 10^{-5}, then even in a relatively small population on the order of 2,000, the selection coefficient of 10^{-3} would be large enough to overcome drift and cause fixation of the canalizing gene. Of course, the maximum selection coefficients that we have derived here could only be realized if robustness was perfect, so larger population sizes are probably required for more realistic levels of robustness.

Migration and Genetic Canalization

While mutation load can play a role in altering large genetic networks, migration load can have a significant effect even when local adaptation is conferred by a small number of genes. Dominance modifiers can evolve when a single locus is in migration selection balance, allowing the de novo creation of a two-gene network. In a scenario where there is mixing between two habitats, Otto and Bourguet (1999) have shown that dominance modifiers that favor the allele with the highest reproductive value will evolve. Likewise, the mainland-island approach taken here allows the evolution of dominance modifiers, suggesting that spatial heterogeneity may generally result in genetic canalization. Thus, spatial heterogeneity may be responsible for the observation of genetically canalized networks, and canalization of the mutation load may simply be a by-product.

Recent work on the evolution and maintenance of polymorphism in a spatial context has suggested that spatial polymorphism should be more common than was previously thought. This is because traditional population genetic methods considered the allele values that allowed polymorphism while adaptive dynamic techniques consider a continuum of alleles (Kisdi and Geritz 1999; Kisdi 2001). Sexual selection can also play a role in expanding the range of conditions that favor polymorphism by increasing selection on locally adapted alleles (Proulx 1999, 2001, 2002). If spatial polymorphism is expected to be common on theoretical grounds, then the opportunity for the creation of genetic networks through canalization should be high. Two alternative trajectories are possible given this starting point. First, the creation of genetic networks to cope with spatial heterogeneity may, in the long run, reduce genetic diversity. For example, if dominance modifiers evolve to regulate the function of other loci, then the curvature of the fitness function at the adaptation locus will shift, possibly leading back to monomorphism (Kisdi 2001). Second, populations may evolve increased ecological divergence as more genes are recruited to networks that confer local adaptation, leading to the buildup of incompatibility between habitats and setting the stage for reinforcement and speciation (Dobzhansky 1940; Muller 1942; Kirkpatrick and Servedio 1999; Servedio 2000).

Phenotypic Plasticity and Canalization

The concepts of canalization and phenotypic plasticity are often discussed together and are tightly linked (for a review, see Debat and David 2001), and at some level environmental canalization must produce phenotypic plasticity (de Visser et al. 2003). Phenotypic plasticity occurs when a trait varies in response to environmental variation, while environmental canalization evolves by reducing the variance in a phenotype when the organism is exposed to environmental variance. These concepts would seem to be opposed but in fact share some important features. To begin, we must consider a point in time where the trait in question does respond to environmental variance: in other words, the trait begins as phenotypically plastic. For canalization to occur, this phenotypic plasticity at the trait level must be converted to a form of phenotypic plasticity at an underlying level. In order for development to produce a final trait that no longer responds to the environment, some component of development must have changed, as compared with the ancestral condition.

This idea can be illustrated through an example of the control of adult body size in insects. Adult body size depends on growth rate during the larval stage and the amount of time spent in development (Nylin and Gotthard 1999). If we manipulate the temperature of a developing insect, the growth rate will change, and if the time of pupation is held constant, then adult body size will show phenotypic plasticity. Canalization to this environmental variation can be achieved by altering the timing of pupation so that constant adult body size is maintained. Thus, the variability of adult body size can be converted

into variability in the timing of pupation and canalization of body size. It seems likely that in any developmental system, canalization of any adult trait must occur through plasticity of some underlying developmental stage. In general, environmental canalization must produce phenotypic plasticity at some level.

Must plasticity also imply canalization? While phenotypic plasticity in general need not lead to canalization, adaptive phenotypic plasticity must. Adaptive phenotypic plasticity is defined as variation in a trait that increases fitness when compared to other fixed phenotypes (Via et al. 1995). In order for fitness to be increased in plastic individuals, then the mean fitness averaged over the distribution of environments encountered must be higher for the plastic types as compared to all other fixed phenotypes. If fitness is defined as a function of the phenotype only, then the variance in fitness will decrease as adaptive plasticity evolves. This means that some life-history variables must show less variance in that they produce lifetime fitness. Thus, adaptive phenotypic plasticity necessarily implies that some trait is canalized, and environmental canalization at the level of an observed trait is likely to involve plasticity at some underlying level. This means that the same genetic features that are associated with adaptive plasticity at one level will be associated with environmental canalization at another level.

Final Thoughts

These results highlight the fact that ecological interactions play an important role in shaping genetic systems. The idea that environmental effects might play a dominant role in the evolution of canalization has been gaining ground in recent years (Wagner et al. 1997; Gibson and Wagner 2000; Meiklejohn and Hartl 2002; Stearns 2002; Milton et al. 2003), and our framework allows a direct comparison of the opportunity for canalization afforded by distinct perturbations. A completely genetic explanation for the evolution of genetic networks through canalization seems unlikely unless either a single gene can influence the effect of mutations at many other loci simultaneously or overdominance commonly evolves. On the other hand, both temporally and spatially varying selection can produce strong selection for canalization and promote the addition of regulatory genes to existing networks. Such environmental influences may shape genetic network evolution even when the genetic interactions themselves can not. This suggests an addition to Dobzhansky's (1973) famous quote: nothing in genetics makes complete sense except in the light of ecology.

Acknowledgments

We thank J. Hermisson and S. Otto for valuable comments that greatly improved this article. S.R.P. was supported by National Institutes of Health (NIH) fellowship GM068382, and P.C.P. was supported by NIH grant GM54185.

Literature Cited

Ancel, L. W., and W. Fontana. 2000. Plasticity, evolvability, and modularity in RNA. Journal of Experimental Zoology 288:242–283.

Barkai, N., and S. Leibler. 1997. Robustness in simple biochemical networks. Nature 387:913–917.

Bergman, A., and M. L. Siegal. 2003. Evolutionary capacitance as a general feature of complex gene networks. Nature 424:549–552.

Bourguet, D. 1999. The evolution of dominance. Heredity 83:1–4.

Bulmer, M. G. 1984. Risk avoidance and nesting strategies. Journal of Theoretical Biology 106:529–535.

Burger, R. 2000. The mathematical theory of selection, recombination, and mutation. Wiley, Chichester.

Caswell, H. 2001. Matrix population models: construction, analysis, and interpretation. 2nd ed. Sinauer, Sunderland, MA.

Charlesworth, D., M. T. Morgan, and B. Charlesworth. 1990. Inbreeding depression, genetic load and the evolution of outcrossing rates in a multi-locus system with no linkage. Evolution 44:1469–1489.

Cherry, J. L. 2002. Deleterious mutation and the evolution of eusociality. Evolution 56:2359–2367.

Chesson, P. L., and S. Ellner. 1989. Invasibility and stochastic boundedness in monotonic competition models. Journal of Mathematical Biology 27:117–138.

Chesson, P. L., and R. R. Warner. 1981. Environmental variability promotes coexistence in lottery competitive systems. American Naturalist 117:923–943.

Clark, A. G. 1994. Invasion and maintenance of a gene duplication. Proceedings of the National Academy of Sciences of the USA 91: 2950–2954.

Crow, J. F. 1958. Some possibilities for measuring selection intensities in man. Human Biology 30:1–13.

———. 1970. Genetic loads and the cost of natural selection. Pages 128–177 *in* K. I. Kojima, ed. Mathematical models in population genetics. Springer, Berlin.

———. 1993. Mutation, mean fitness, and genetic load. Pages 3–4 *in* R. Dawkins and M. Ridley, eds. Oxford surveys in evolutionary biology. Vol. 9. Oxford University Press, Oxford.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper & Row, New York.

Debat, V., and P. David. 2001. Mapping phenotypes: canalization, plasticity and developmental stability. Trends in Ecology & Evolution 16:555–561.

Dempster, E. R. 1955. Maintenance of genetic heterogeneity. Cold Spring Harbor Symposia on Quantitative Biology 20:25–32.

de Visser, J. A. G. M., J. Hermisson, G. P. Wagner, L. A. Meyers, H. Bagheri-Chaichian, J. L. Blanchard, L. Chao, et al. 2003. Perspective: evolution and detection of genetic robustness. Evolution 57:1959–1972.

Dieckmann, U., and R. Law. 1996. The dynamical theory of coevolution: a derivation from stochastic ecological processes. Journal of Mathematical Biology 34:579–612.

Dobzhansky, T. 1955. A review of some fundamental concepts and

problems of population genetics. Cold Spring Harbor Symposia on Quantitative Biology 20:1–15.

———. 1973. Nothing in biology makes sense except in the light of evolution. American Biology Teacher 35:125–129.

Dobzhansky, T. H. 1940. Speciation as a stage in evolutionary divergence. American Naturalist 74:312–321.

Eigen, M., and P. Schuster. 1977. The hypercycle: a principle of natural self-organization. Naturwissenschaften 64:541–565.

Ellner, S. 1984. Asymptotic behavior of some stochastic difference equation population models. Journal of Mathematical Biology 19:169–200.

Ellner, S. P. 1996. You bet your life: life-history strategies in fluctuating environments. Pages 3–24 *in* H. G. Othmer, F. R. Adler, M. A. Lewis, and J. C. Dallon, eds. Case studies in mathematical modeling: ecology, physiology and cell biology. Prentice Hall, Upper Saddle River, NJ.

Eshel, I., and C. Matessi. 1998. Canalization, genetic assimilation and preadaptation: a quantitative genetic model. Genetics 149:2119–2133.

Feldman, M. W., and S. Karlin. 1971. The evolution of dominance: a direct approach through the theory of linkage and selection. Theoretical Population Biology 2:482–492.

Fell, D. 1997. Frontiers in metabolism. Vol. 2. Understanding the control of metabolism. Portland, London.

Fisher, R. A. 1928. The possible modification of the response of the wild type to recurrent mutations. American Naturalist 62:115–126.

———. 1958. The genetical theory of natural selection. Dover, New York.

Frank, S. A., and M. Slatkin. 1990. Evolution in a variable environment. American Naturalist 136:244–260.

Furlong, E. E. M., E. C. Andersen, B. Null, K. P. White, and M. P. Scott. 2001. Patterns of gene expression during *Drosophila* mesoderm development. Science 293:1629–1633.

Gavrilets, S., and A. Hastings. 1994. A quantitative-genetic model for selection on developmental noise. Evolution 48:1478–1486.

Gibson, G., and G. Wagner. 2000. Canalization in evolutionary genetics: a stabilizing theory? BioEssays 22:372–380.

Gillespie, J. H. 1973. Natural selection with varying selection coefficients: a haploid model. Genetical Research 21:115–120.

———. 1974. Natural selection for within generation variance in offspring number. Genetics 76:601–606.

Haldane, J. B. S. 1930. A note on Fisher's theory of the origin of dominance, and on a correlation between dominance and linkage. American Naturalist 63:87–90.

———. 1937. The effect of variation on fitness. American Naturalist 71:337–349.

Hammerstein, P. 1996. Darwinian adaptation, population genetics and the streetcar theory of evolution. Journal of Mathematical Biology 34:511–532.

Hansen, T. F. 2003. Is modularity necessary for evolvability? remarks on the relationship between pleiotropy and evolvability. Biosystems 69:83–94.

Hansen, T. F., and G. P. Wagner. 2001. Modeling genetic architecture: a multilinear model of gene interaction. Theoretical Population Biology 59:61–86.

Hermisson, J., O. Redner, H. Wagner, and E. Baake. 2002. Mutation-selection balance: ancestry, load, and maximum principle. Theoretical Population Biology 62:9–46.

Hermisson, J., T. F. Hansen, and G. P. Wagner. 2003. Epistasis in polygenic traits and the evolution of genetic architecture under stabilizing selection. American Naturalist 161:708–734.

Hurst, L. D., and J. P. Randerson. 2000. Dosage, deletions and dominance: simple models of the evolution of gene expression. Journal of Theoretical Biology 205:641–647.

Ideker, T., V. Thorsson, J. A. Ranish, R. Christmas, J. Buhler, J. K. Eng, R. Bumgarner, D. R. Goodlett, R. Aeberold, and L. Hood. 2001. Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. Science 292:929–934.

Johnson, T. 1999. The approach to mutation-selection balance in an infinite asexual population, and the evolution of mutation rates. Proceedings of the Royal Society of London B 266:2389–2397.

Kaplan, R. H., and W. S. Cooper. 1984. The evolution of developmental plasticity in reproductive characteristics: an application of the "adaptive coin-flipping" principle. American Naturalist 123:393–410.

Karlin, S., and M. W. Feldman. 1970. Linkage and selection: two locus symmetric viability model. Theoretical Population Biology 1:39–71.

Kawecki, T. J. 2000. The evolution of genetic canalization under fluctuating selection. Evolution 54:1–12.

Keightley, P. D. 1996. A metabolic basis for dominance and recessivity. Genetics 143:621–625.

King, J. L. 1966. The gene interaction component of the genetic load. Genetics 53:403–413.

Kirkpatrick, M., and M. R. Servedio. 1999. The reinforcement of mating preferences on an island. Genetics 151:865–884.

Kisdi, E. 2001. Long-term adaptive diversity in Levene-type models. Evolutionary Ecology Research 3:721–727.

Kisdi, E., and S. A. H. Geritz. 1999. Adaptive dynamics in allele space: evolution of genetic polymorphism by small mutations in a heterogeneous environment. Evolution 53:993–1008.

Kondrashov, A. S. 1984. Deleterious mutations as an evolutionary factor. I. The advantage of recombination. Genetical Research 44:199–217.

Kondrashov, A. S., and J. F. Crow. 1988. King's formula for the mutation load with epistasis. Genetics 120:853–856.

Lande, R. 1976. Natural selection and random genetic drift in phenotypic evolution. Evolution 30:314–334.

Lee, T. I., N. J. Rinaldi, F. Robert, D. T. Odom, Z. Bar-Joseph, G. K. Gerber, N. M. Hannett, et al. 2002. Transcriptional regulatory networks in *Saccharomyces cerevisiae*. Science 298:799–804.

Lerner, I. M. 1954. Genetic homeostasis. Oliver & Boyd, London.

Levins, R. 1962. Theory of fitness in a heterogeneous environment. I. The fitness set and adaptive function. American Naturalist 96:361–373.

Lipson, H., J. B. Pollack, and N. P. Suh. 2002. On the origin of modular variation. Evolution 56:1549–1556.

Maynard Smith, J. 1982. Evolution and the theory of games. Cambridge University Press, Cambridge.

Mayr, E. 1960. The emergence of evolutionary novelties. Pages 349–380 *in* S. Tax, ed. Evolution after Darwin. Vol. 1. The evolution of life. University of Chicago Press, Chicago.

Meiklejohn, C. D., and D. L. Hartl. 2002. A single mode of canalization. Trends in Ecology & Evolution 17:468–473.

Metz, J. A. J., R. M. Nisbet, and S. A. H. Geritz. 1992. How should we define "fitness" for general ecological scenarios. Trends in Ecology & Evolution 7:198–202.

Milton, C. C., B. Huynh, P. Batterham, S. L. Rutherford, and A. A. Hoffmann. 2003. Quantitative trait symmetry independent of Hsp90 buffering: distinct modes of genetic canalization and developmental

stability. Proceedings of the National Academy of Sciences of the USA 100:13396–13401.
Muller, H. J. 1942. Isolating mechanisms, evolution and temperature. Biological Symposia 6:71–125.
———. 1950. Our load of mutations. American Journal of Human Genetics 2:111–176.
Nagylaki, T. 1977. Lecture notes in biomathematics. Vol. 15. Selection in one- and two-locus systems. Springer, New York.
Nowak, M., M. Boerlijst, J. Cooke, and J. Smith. 1997. Evolution of genetic redundancy. Nature 388:167–171.
Nowak, M. A. 1992. What is a quasispecies? Trends in Ecology & Evolution 7:118–121.
Nylin, S., and K. Gotthard. 1999. Plasticity in life-history traits. Annual Review in Entomology 43:63–83.
Omholt, S., E. Plahte, L. Oyehaug, and K. Xiang. 2000. Gene regulatory networks generating the phenomena of additivity, dominance and epistasis. Genetics 155:969–980.
Otto, S. P., and D. Bourguet. 1999. Balanced polymorphisms and the evolution of dominance. American Naturalist 153:561–574.
Otto, S. P., and J. Whitton. 2000. Polyploid incidence and evolution. Annual Review of Genetics 34:401–437.
Otto, S. P., and P. Yong. 2002. The evolution of gene duplicates. Pages 451–483 *in* J. C. Dunlap and C. Wu, eds. Advances in genetics. Vol. 46. Homology effects. Academic Press, San Diego, CA.
Pál, C., and L. D. Hurst. 2000. The evolution of gene number: are heritable and non-heritable errors equally important. Heredity 84: 393–400.
Phillips, P., and N. Johnson. 1998. The population genetics of synthetic lethals. Genetics 150:449–458.
Phillips, P. C., S. P. Otto, and M. S. Whitlock. 2000. Beyond the average: the evolutionary importance of gene interactions and variability of epistatic effects. Pages 20–38 *in* J. B. Wolf, E. D. Brodie III, and M. J. Wade, eds. Epistasis and the evolutionary process. Oxford University Press, Oxford.
Price, G. R. 1970. Selection and covariance. Nature 227:529–531.
Proulx, S. R. 1999. Matings systems and the evolution of the niche. American Naturalist 154:89–98.
———. 2000. The ESS under spatial variation with applications to sex allocation. Theoretical Population Biology 58:33–47.
———. 2001. Female choice via indicator traits easily evolves in the face of recombination and migration. Evolution 55:2401–2411.
———. 2002. Niche shifts and expansion due to sexual selection. Evolutionary Ecology Research 4:351–369.
Proulx, S. R., and T. Day. 2001. What can invasion analyses tell us about evolution under stochasticity in finite populations? Selection: Molecules, Genes, and Memes 2:2–15.
Rice, S. H. 1998. The evolution of canalization and the breaking of von Baer's laws: modeling the evolution of development with epistasis. Evolution 52:647–656.
Rutherford, S. L., and S. Lindquist. 1998. HSP90 as a capacitor for morphological evolution. Nature 396:336–342.
Schmalhausen, I. I. 1949. Factors of evolution. Blakiston, Philadelphia.
Seger, J., and H. J. Brockmann. 1987. What is bet-hedging? Pages 182–211 *in* R. Dawkins and M. Ridley, eds. Oxford surveys in evolutionary biology. Vol. 4. Oxford University Press, Oxford.
Servedio, M. R. 2000. Reinforcement and the genetics of nonrandom mating. Evolution 54:21–29.
Shaw, R. F., and J. D. Mohler. 1953. The selective significance of the sex ratio. American Naturalist 87:337–342.
Siegal, M., and A. Bergman. 2002. Waddington's canalization revisited: developmental stability and evolution. Proceedings of the National Academy of Sciences of the USA 99:10528–10532.
Sniegowski, P. D., P. J. Gerrish, T. Johnson, and A. Shaver. 2000. The evolution of mutation rates: separating causes from consequences. BioEssays 22:1057–1066.
Stearns, S. C. 2002. Progress on canalization. Proceedings of the National Academy of Sciences of the USA 99:10229–10230.
Taylor, P., and T. Day. 1997. Evolutionary stability under the replicator and the gradient dynamics. Evolutionary Ecology 11:579–590.
Tuljapurkar, S. 1990. An uncertain life: demography in random environments. Theoretical Population Biology 35:227–294.
Uetz, P., L. Giot, G. Cagney, T. A. Manseld, R. S. Judson, J. R. Knight, D. Lockshon, et al. 2000. A comprehensive analysis of protein-protein interactions. Nature 403:623–627.
van Nimwegen, E., J. P. Crutchfield, and M. Huynen. 1999. Neutral evolution of mutational robustness. Proceedings of the National Academy of Sciences of the USA 96:9716–9720.
Via, S., R. Gomulkiewicz, G. de Jong, S. Scheiner, C. D. Schlichting, and P. H. van Tienderen. 1995. Adaptive phenotypic plasticity: consensus and controversy. Trends in Ecology & Evolution 5:212–217.
von Dassow, G., E. Meir, E. M. Munro, and G. M. Odell. 2000. The segment polarity network is a robust developmental module. Nature 406:188–192.
Waddington, C. H. 1942. The canalization of development and genetic assimilation of acquired characters. Nature 150:563–565.
Wagner, A. 1996. Does evolutionary plasticity evolve? Evolution 50: 1008–1023.
———. 1999. Redundant gene functions and natural selection. Journal of Evolutionary Biology 12:1–16.
———. 2003. How the global structure of protein interaction networks evolves. Proceedings of the Royal Society of London B 270:457–466.
Wagner, A., and D. A. Fell. 2001. The small world inside large metabolic networks. Proceedings of the Royal Society of London B 268:1803–1810.
Wagner, G. P., and L. Altenberg. 1996. Complex adaptations and the evolution of evolvability. Evolution 50:967–976.
Wagner, G. P., and J. Mezey. 2000. Modeling the evolution of genetic architecture: a continuum of alleles model with pairwise AxA epistasis. Journal of Theoretical Biology 203:163–175.
Wagner, G. P., G. Booth, and H. Bagheri-Chaichian. 1997. A population genetic theory of canalization. Evolution 51:329–347.
Welch, J. J., and D. Waxman. 2003. Modularity and the cost of complexity. Evolution 57:1723–1734.
Whitlock, M. C. 2002. Selection, load, and inbreeding depression in a large metapopulation. Genetics 160:1191–1202.
Wilke, C. O., J. L. Wang, C. Ofria, R. E. Lenski, and C. Adami. 2001. Evolution of digital organisms at high mutation rates leads to survival of the flattest. Nature 412:331–333.
Wright, S. 1929. Fisher's theory of dominance. American Naturalist 63:274–279.
———. 1931. Evolution in Mendelian populations. Genetics 16:97–159.
———. 1934. Physiological and evolutionary theories of dominance. American Naturalist 64:24–53.
Yoshimura, J., and C. W. Clark. 1991. Individual adaptations in stochastic environments. Evolutionary Ecology 5:173–192.

Associate Editor: Günter P. Wagner