Evol Biol (2013) 40:317–340
DOI 10.1007/s11692-012-9218-z

RESEARCH ARTICLE

The Evolution of Canalization and Evolvability in Stable and Fluctuating Environments

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Received: 7 November 2012 / Accepted: 10 December 2012 / Published online: 6 January 2013

Abstract Using a multilinear model of epistasis we explore the evolution of canalization (reduced mutational effects) and evolvability (levels of additive genetic variance) under different forms of stabilizing and fluctuating selection. We show that the total selection acting on an allele can be divided into a component deriving from adaptation of the trait mean, a component of canalizing selection favoring alleles that epistatically reduce the effects of other allele substitutions, and a component of conservative selection disfavoring rare alleles. While canalizing selection operates in both stable and fluctuating environments, it may not typically maximize canalization, because it gets less efficient with increasing canalization, and reaches a balance with drift, mutation and indirect selection. Fluctuating selection leads to less canalized equilibria than stabilizing selection of comparable strength, because canalization then becomes influenced by erratic correlated responses to shifting trait adaptation. We conclude that epistatic systems under bounded fluctuating selection will become less canalized than under stabilizing selection and may support moderately increased evolvability if the amplitude of fluctuations is large, but canalization is still stronger and evolvability lower than expected under neutral evolution or under patterns of selection that shift the trait in directions of positive (reinforcing) epistasis.

Keywords Canalization · Evolvability · Epistasis · Mutation-selection balance · Fluctuating selection

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Introduction

Field studies indicate that many traits routinely experience strong directional selection (Hereford et al. 2004; Bell 2010), and undergo rapid evolutionary changes (Hendry and Kinnison 1999; Kinnison and Hendry 2001; Hendry et al. 2008). Short-term evolutionary changes are usually not converted into permanent evolutionary diversification however. In fact, the cumulative amount of evolutionary change observed over as much as a million generations is about the same as the cumulative change observed over just a few generations (Gingerich 1983; Estes and Arnold 2007; Uyeda et al. 2011). Only on time scales involving millions of years does regular cumulative evolution become common (Uyeda et al. 2011). These patterns seem paradoxical and although several hypotheses have been proposed to explain them, we still lack a clear understanding of why frequent short-term selection and microevolution are not typically converted into macroevolutionary change (Futuyma 1987, 2010; Williams 1992; Arnold et al. 2001; Gould 2002; Hansen and Houle 2004; Estes and Arnold 2007; Hansen 2012).

One possible explanation is that observed selection is caused by rapid but constrained fluctuations of the selective environment, so that selection is effectively stabilizing on longer time scales. This is consistent with frequent observations of spatial and temporal fluctuations in directional

selection (reviewed in Gosden and Svensson 2008; Hereford 2009; Siepielski et al. 2009; Bell 2010; Calsbeek et al. 2012; but see Morrissey and Hadfield 2012). While it remains unclear why selective optima or the ability of organisms to track them may be constrained to a fixed range for millions of generations (Futuyma 2010; Hansen 2012), it is likely that rapid, constrained fluctuations constitute a common mode of microevolution.

For this reason it is pertinent to develop the theoretical and empirical understanding of the maintenance of evolvability under fluctuating selection. While there seems to be a loose consensus that fluctuating selection favors the evolution of evolvability (e.g. Wagner 1996; Wagner and Altenberg 1996; Kawecki 2000; Flatt 2005; Lee and Gelembiuk 2008), there is little formal theoretical work on this question. There is a body of work on the maintenance of genetic variance by mutation under fluctuating selection in additive models, but this has yielded mixed results where genetic variation can be either elevated, reduced or remain unchanged relative to stabilizing selection (e.g. Felsenstein 1976; Lande 1977; Bürger and Lynch 1995; Kondrashov and Yampolsky 1996; Sasaki and Ellner 1997; Bürger 1999; Bürger and Gimelfarb 2002; Jones et al. 2004, 2012; Revell 2007; Svardal et al. 2011; this paper). It is also well known that fluctuating selection can maintain single-locus variation by creating protected polymorphisms, as intermediate heterozygotes tend to have the highest multiplicative fitness (Haldane and Jayakar 1963; Karlin and Liberman 1974; Gillespie 1991), but so can happen under stabilizing selection as well, and any relevance for polygenic traits is unclear.

The maintenance of genetic variation depends on facts of the genetic architecture such as gene number, mutational effects and epistasis, and to understand the evolution of evolvability we need to understand how this architecture evolves (Wagner and Altenberg 1996; Hansen 2006; Mitteroecker 2009). Waddington's (1942) concept of canalization stands central. In population-genetic terms, canalization is understood as the evolution of reduced allelic and mutational effects (Wagner et al. 1997). Waddington (1942, 1957) hypothesized that this would be favored under stabilizing selection, and conversely, one can imagine decanalization as an adaptation for increased evolvability in unstable environments. There is a well-established finding that stress and environmental change often lead to decanalization and increase of genetic variation (Scharloo 1991; Moreno 1994; Hoffmann and Parsons 1997; Flatt 2005). This has been interpreted as an adaptation to increase evolvability when the environment is changing (e.g., Rutherford and Lindquist 1998), but can also be explained as an automatic outcome of random changes in an epistatic genetic architecture (Wagner and Mezey 2000; Hansen and Wagner 2001a; Hermisson and Wagner 2004; Zhang 2008; Zhang and Hill 2010).

Indeed, much literature on the evolution of genetic architecture is centered on the idea that genetic systems evolve as adaptations for evolvability or robustness (e.g. Riedl 1978; Wagner and Altenberg 1996; Gerhart and Kirchner 1997; Earl and Deem 2004; Pavlicev et al. 2011). It is not obvious, however, that we should expect species in unstable environments to become more evolvable, because the direct selection pressures necessary to produce such adaptations are often weak (Wagner and Bürger 1985; Proulx and Phillips 2005; Jones et al. 2007), and therefore vulnerable to be overcome by genetic drift (Lynch 2007a, b) or by indirect selection resulting from trait adaptation (Hansen 2011). The role of indirect selection is well illustrated by the results of Carter et al. (2005), who showed that canalization and evolvability could either increase, decrease, or remain roughly constant under directional selection depending on the pattern of epistatic interactions. If genes affecting the trait or trait combination under selection tend to have positive epistatic effects on each other, then a rapid decanalization and increase of evolvability can ensue, but this would be due to indirect selection stemming from selection on the trait, and should not be considered an adaptation for evolvability unless this pattern of epistasis itself was a such adaptation. Counteradaptive canalization can also happen if gene substitutions with positive effects on the trait tend to have negative epistatic interactions that reduce the effects of subsequent gene substitutions. In this case evolution of the trait can come to a standstill, and although Hansen et al. (2006) showed that such epistatic constraints eventually can be broken and evolvability restored, this may take tens of thousands of generations or more. In either case, evolution of the genetic architecture is driven by trait adaptation, and changes in variational properties as evolvability occur as correlated responses that may go in any direction depending on the details of the genotype–phenotype map.

Stabilizing selection keeps the trait mean constant, and selection on variational properties may therefore exert itself more than under directional selection. Stabilizing selection disfavors variation (Wright 1935; Bulmer 1971; Layzer 1980), and it can be shown analytically that this generates canalizing selection in favor of genotypes that reduce expressed genetic (or environmental) variation (Slatkin and Lande 1976; Gavrilets and Hastings 1994; Wagner et al. 1997; Rice 1998, 2002; Hermisson et al. 2003; Zhang 2005; Zhang and Hill 2005, 2008; Kopp and Hermisson 2006; Álvarez-Castro et al. 2009). The dynamics are complicated, however, because the canalization of different genes may conflict and must happen within the constraint of keeping the trait at the optimum. Stabilizing selection is also a conservative force, disfavoring rare alleles with large average effects, and since evolution of canalization depends on quasi-neutral

substitution of alleles, it may slow down and stop if stabilizing selection gets too strong relative to the segregating allelic effect sizes (Fierst and Hansen 2010). Strong stabilizing selection may also weaken canalizing selection by reducing segregating variation, and traits under intermediate-strength stabilizing selection may experience the strongest canalization (Wagner et al. 1997). Furthermore, although canalization will reduce segregating trait variance (at least in multilinear epistatic systems; Hermisson et al. 2003), it also allows the accumulation of molecular variation that is hidden on the trait level, and such hidden variation could be released to boost evolvability if an environmental change causes genetic changes due to selection on the trait mean (Waddington 1953; Eshel and Matessi 1998; Rutherford 2000; Hermisson and Wagner 2004; Masel 2005; Hansen et al. 2006; Le Rouzic and Carlborg 2008; Zhang 2008).

Many types of rapid fluctuating selection result in long-term stabilizing multiplicative selection, and can be expected to have similar consequences as constant stabilizing selection. Even so, canalization could still be influenced by a shifting trait mean or shifting convexity of the fitness function. So far, the only study that has focused explicitly on genetic canalization under fluctuating selection is by Kawecki (2000). Using simple modifier models, he found that canalizing alleles were favored under fluctuations with very short period, but there was a tendency for polymorphism or decanalization when fluctuations reached periodicities above ten generations. Some studies focused on the evolution of modularity have also found a tendency for evolvability to be enhanced under fluctuating selection (e.g., Jones et al. 2007; Draghi and Wagner 2008, 2009; Fierst 2011). Yet, the mechanisms behind and generality of such results are unclear. Finally, it has been found that large-amplitude fluctuations may favor the evolution of environmental decanalization and plasticity (e.g., Slatkin and Lande 1976; Bull 1987; Zhang and Hill 2005; Lande 2009; Svardal et al. 2011).

Taken together, these results motivate the hypothesis that fluctuating selection may elevate evolvability above levels expected under stabilizing selection, but the exact mechanisms are poorly understood, and may involve the detailed architecture of both genetics and fluctuations. Here we attempt a systematic investigation of the effects of temporal fluctuations on an evolvable genetic system with particular emphasis on understanding the general evolutionary forces acting on gene effects. This is a part of a long-term project to understand the evolution of evolvability and canalization by use of the multilinear epistatic model of Hansen and Wagner (2001a), which provides a relatively general, yet transparent and analytically tractable model for the evolution of gene effects. We have previously looked in detail at the effects of directional (Hansen and Wagner 2001b; Carter et al. 2005; Hansen et al. 2006; Pavlicev et al. 2010) and stabilizing selection (Hermisson et al. 2003; Álvarez-Castro et al. 2009; Fierst and Hansen 2010). Here we extend this to fluctuating selection. The main hypothesis we test is whether environmental fluctuations can generate systematic changes in the genotype–phenotype map that could change its capacity to produce and maintain genetic variation and hence its evolvability.

Theory

Fitness in a Fluctuating Environment

Let $W(z;t)$ be the absolute fitness of an individual with trait z in generation t. Then the effects of selection over T generations can be represented by the multiplicative relative fitness $\prod_t W(z;t)/\bar{W}(t)$, where t runs from 1 to T, and $\bar{W}(t)$ is the mean fitness in generation t. If transmission is perfect, the frequency of type z after these T episodes can be found by multiplying its initial frequency with the multiplicative relative fitness (Dempster 1955; Levins 1962; Haldane and Jayakar 1963; Cohen 1966). More specifically, the frequency, p_T, after T generations of an allele with initial frequency p_0 will be $p_0 \prod_t W(\bar{z}+x;t)/\bar{W}(t)$, where x is the allelic effect. This assumes that allele frequencies change deterministically. If they behave stochastically according to a diffusion process, a modified fitness taking account of stochastic covariance between changes in allele frequencies and mean fitness is appropriate (Lande 2007, 2008).

The effects of selection on variance depend on the convexity of the fitness function (e.g. Layzer 1980). Hence, we start by assessing the convexity of multiplicative fitness. The first and second derivatives of the multiplicative relative fitness function are

$$\partial \prod_t \frac{W(z;t)}{\bar{W}(t)} \Big/ \partial z = \prod_t \frac{W(z;t)}{\bar{W}(t)} \Sigma_t \frac{\partial W(z;t)/\partial z}{W(z;t)}, \tag{1a}$$

$$\partial^2 \prod_t \frac{W(z;t)}{\bar{W}(t)} \Big/ \partial z^2 = \prod_t \frac{W(z;t)}{\bar{W}(t)} \left[\Sigma_t \frac{\partial^2 W(z;t)/\partial z^2}{W(z;t)} - \Sigma_t \left(\frac{\partial W(z;t)/\partial z}{W(z;t)} \right)^2 + \left(\Sigma_t \frac{\partial W(z;t)/\partial z}{W(z;t)} \right)^2 \right]. \tag{1b}$$

An extremum exists where the sum in Eq. 1a is zero. The convexity of the fitness landscape is described by Eq. 1b. For a heuristic interpretation we assume that the trait mean is in the vicinity of the long-term optimum defined by setting Eq. 1a equal to zero and that there is little variation in the trait so that we can make the approximation $\bar{W}(t) \approx W(\bar{z};t)$. With these assumptions the second derivative at the long-term optimum is approximately

$$\partial^2 \prod_t \frac{W(\bar{z};t)}{\bar{W}(t)} \Big/ \partial z^2 \approx E_t\left[\frac{\partial^2 W(\bar{z};t)/\partial z^2}{W(\bar{z};t)}\right] T - Var_t\left[\frac{\partial W(\bar{z};t)/\partial z}{W(\bar{z};t)}\right] T, \quad (2)$$

where the expectation and the variance are taken over T generations. Hence, the convexity of multiplicative relative fitness is approximately the sum of the average within-generation convexity around the trait mean, and (minus) the variance of the (linear) selection gradients across generations. This shows that a fluctuating selection gradient (the variance term) will tend to make the population experience cumulative stabilizing selection that is stronger than the average within-generation stabilizing selection. Even so, this does not necessarily mean stronger stabilizing selection, since fluctuations could also make the population experience more convex areas of the fitness landscape and thus increase the average-convexity term.

In "Appendix 1" we explore this trade off for some specific fitness functions and patterns of fluctuation. For a Gaussian fitness function with a stationary fluctuating optimum, we show that these effects exactly cancel and the multiplicative fitness function is also Gaussian with the same strength of stabilizing selection regardless of the size of fluctuations in the optimum. A quadratic fitness function with a fluctuating optimum, however, will produce slightly stronger multiplicative stabilizing selection since its curvature is constant. Likewise, fluctuating linear stabilizing selection will produce a multiplicative fitness landscape that is either stabilizing or "normalizing" (i.e. concave around trait mean; Travis 1989). Fluctuating exponential directional selection, on the other hand is sufficiently convex to produce a multiplicative fitness landscape that is flat, so that a form of neutral drift can ensue.

In summary, there is a tendency for stationary fluctuating selection to cumulatively act stabilizing or at least normalizing. This means that fluctuating selection is inherently disfavorable to variation in a way that is similar to pure stabilizing selection. The exact effects may depend, however, on the distribution of fluctuations in relation to patterns of convexity in the fitness landscape, and on how the mean phenotype is changing during fluctuations. There is also a complex relation between selection and evolution of variation, which depends on details of genetic architecture. We proceed to investigate this in detail.

The Multilinear Genotype–Phenotype Map

The multilinear epistatic model is based on the assumption that a substitution on one locus can alter the effect of any genetic substitution at other loci, but only as a linear function of its own effect (Hansen and Wagner 2001a). If we represent the genotype, $g = \{{}^1y, \ldots, {}^ny\}$, as a set of reference effects, ${}^iy$, of loci $i = 1, \ldots, n$, each defined as the effect of substituting the genotype at this locus into a chosen reference genotype, then the (expected) phenotype, z, associated with g becomes a multilinear function

$$z = z_r + \Sigma_i {}^iy + \Sigma_{j>i} {}^{ij}\varepsilon {}^iy {}^jy + [\text{higher-order terms}], \quad (3)$$

where z_r is the (expected) phenotype of the reference genotype and summations run over all loci in g. The ${}^{ij}\varepsilon$ are epistasis coefficients describing the interaction between loci i and j. Note that ${}^{ii}\varepsilon = 0$ and ${}^{ij}\varepsilon = {}^{ji}\varepsilon$. We can see that a change ${}^iy$ from the reference genotype at locus i will canalize locus j, in the sense of reducing the phenotypic difference between any genotypes at this locus, precisely when ${}^{ij}\varepsilon {}^iy$ is in the interval $(-2, 0)$. Whether a whole genotype, g, canalizes a particular locus, i, relative to the reference genotype is described by the epistasis factor

$${}^{g\to i}f = 1 + \Sigma_j {}^{ij}\varepsilon {}^jy + [\text{higher-order terms}], \quad (4)$$

where j runs over all loci in g. If this factor is less than one in absolute value the locus gets canalized by g, and if it is larger than one the locus gets decanalized by g. Note that ${}^{g\to i}f = 1$ means no change, ${}^{g\to i}f = 0$ means complete canalization, and ${}^{g\to i}f < 0$ means that the sign of effects is flipped so that previously positive changes become negative changes.

The multilinear model is thus an extension of the additive model that allows gene and genotype effects to evolve in a simple manner. It can be regarded as a local approximation of the genotype–phenotype map around the chosen reference genotype. Note that all variables and parameters are defined relative to this specific reference, and may change if a different reference genotype is used. Specific formulas to describe these changes are given in Hansen and Wagner (2001a). Hence, if predictions of the model are to be compared against empirical estimates of epistasis and gene effects, it is essential to consider what empirical reference genotype was used (see Barton and Turelli 2004; Álvarez-Castro and Carlborg 2007; Le Rouzic and Álvarez-Castro 2008; Álvarez-Castro and Yang 2012 for details and generalizations).

Selection on Canalization

In this section, we present analytical expressions for the strength and direction of selection on alleles with (de)canalizing effects. We start by picking out one locus in the model and label its reference effect as x, then with pairwise epistasis, the expected phenotype is

$$z = z_r + \Sigma_i {}^iy + \Sigma_i \Sigma_{j>i} {}^{ij}\varepsilon {}^iy {}^jy + x + \Sigma_i {}^{ix}\varepsilon {}^iy\, x, \quad (5)$$

where i and j run over all loci except x. A general model with higher-order epistasis is analyzed in "Appendix 2". To

understand how selection acts on x, we compare two genotypes with values $x = 0$ and $x = 1$, which simply means that x sets the scale. The coefficients ${}^{ix}\varepsilon$ then measure the strength and direction of canalization caused by substituting $x = 1$ for $x = 0$. If ${}^{ix}\varepsilon \in (-2, 0)$, then this substitution will canalize the ith locus, and if ${}^{ix}\varepsilon \notin [-2, 0]$, the substitution will decanalize the ith locus. Approximating a stabilizing selection function as $W(z) \approx 1 - s(z - \theta)^2$, where $-s/2$ can be thought of as the second derivative in Eq. 2 evaluated at the optimum, and assuming that locus x is in linkage equilibrium with the rest of the genome, the fitness difference between $x = 1$ and $x = 0$ is

$$\begin{aligned}\Delta W &= \mathrm{E}_g[W(z)|x = 1] - \mathrm{E}_g[W(z)|x = 0] \\ &\approx -s\mathrm{E}_g\left[2^{g\to x}f(z_{x=0} - \theta) + {}^{g\to x}f^2\right],\end{aligned} \tag{6}$$

where $z_{x=0} = z_r + \Sigma_i\, {}^{i}y + \Sigma_i\, \Sigma_{j>i}\, {}^{ij}\varepsilon\, {}^{i}y\, {}^{j}y$ is the phenotype of an $x = 0$ genotype and ${}^{g\to x}f = 1 + \Sigma_i\ {}^{ix}\varepsilon\ {}^{i}y$ is the epistasis factor acting on locus x from the rest of the genome, g. Assuming linkage equilibrium, a calculation shown in "Appendix 2" now yields

$$\begin{aligned}\Delta W \approx\ & -2s^{\bar{g}\to x}f(\bar{z} - \theta) - s\Sigma_i\, {}^{ix}\varepsilon\left({}^{ix}\varepsilon + 2s^{\bar{g}\to x}f\right){}^{i}v \\ & - s(1 - 2\bar{x})^{\bar{g}\to x}f^2,\end{aligned} \tag{7}$$

where bars denote population averages, ${}^{i}v = \mathrm{Var}[{}^{i}y]$ is the variance in reference effects of locus i, and we have used the fact that $E_g[{}^{g\to x}f] = {}^{\bar{g}\to x}f$. This equation can be simplified by choosing the genotype $\bar{g} = \{{}^{1}\bar{y}, \ldots, {}^{n}\bar{y}\}$, and $x = 0$ as the reference genotype. With this reference

$$\Delta W \approx -2s(\bar{z} - \theta) - s\Sigma_i\, {}^{ix}\varepsilon\left({}^{ix}\varepsilon + 2\right){}^{i}v - s(1 - 2\bar{x}) \tag{8}$$

Note that ${}^{ix}\varepsilon$, ${}^{i}v$, and x are now dynamic parameters that will change as $\bar{g}$ is changing. The three terms in Eqs. 7 and 8 identify three selective forces on the x-locus.

The first term is the "adaptive force" of Hermisson et al. (2003); it says that the x-locus is selected in whatever direction that brings the trait mean towards the optimum. Whether canalization or decanalization will be favored will then depend on whether it is a shift towards the $x = 0$ or $x = 1$ genotype that brings the mean towards the optimum. Note that this is direct selection and hence an adaptive force in relation to optimizing the trait, but it is indirect selection in relation to canalization and evolvability.

The second term describes canalizing selection acting to reduce variation (cmp. Rice 2002; Hermisson et al. 2003). With ${}^{ix}\varepsilon$ measured in the mean reference (Eq. 8), there will be a positive contribution to fitness for each ${}^{ix}\varepsilon \in (-2, 0)$, i.e. for each locus that is being canalized, and a negative contribution for each locus that is being decanalized. Evaluating the terms with reference to an arbitrary genotype (Eq. 7), we see that there are positive fitness contributions from the effects on loci for which ${}^{ix}\varepsilon$ is between 0 and $-2^{\bar{g}\to x}f$. Hence, on loci that are decanalized by the rest of the genome ($|{}^{\bar{g}\to x}f| > 1$) mutations that are decanalizing in the reference genotype can sometimes be favored, but these would be mutations that decanalize other genes that themselves have a net canalizing effect. We see this by comparing with the effect in the mean reference where a mutation will be favored precisely when it has a net canalizing effect in the average genotype. As loci become more canalized, the mean epistasis factor gets closer to zero and the interval of ${}^{ix}\varepsilon$ values that yields positive fitness effects becomes smaller. This will reduce the rate and size of favorable canalizing mutations, so that the potential for canalizing selection becomes weaker with increasing canalization. This effect will be reinforced if standing "molecular" variances, ${}^{i}v$, are decreasing with decreasing mutational effect sizes (when measured in the mean reference). In the section on mutation-selection balance below, we show that this is the case under "Gaussian" conditions (high mutation rate, small mutational effects), where the ${}^{i}v$ scale approximately linearly with canalization (measured by ${}^{g\to i}f$), but not under "House-of-cards" conditions (low mutation rate, high mutational effects), where the ${}^{i}v$ may even increase with canalization. Note that all this is a result of the concavity of the fitness function, and will be reversed to favor decanalization if the fitness function is convex ($s < 0$).

The third term describes a conservative force that favors any allele near fixation, whether it is canalizing or decanalizing. This is because random changes reduce fitness on average when the fitness function is concave, and rare alleles are disfavored because they have larger average effects (in the quantitative genetics sense of average deviation from population mean). This term will increase in strength relative to the other terms as the strength of stabilizing selection is increasing, because the trait mean is then brought closer to the optimum and genetic variances (${}^{i}v$) are reduced. If stabilizing selection is sufficiently strong, this conservative force may block systems drift (sensu True and Haag 2001) and lead to an evolutionary standstill, as found by Fierst and Hansen (2010).

If the population mean is at a stationary optimum, trait adaptation vanishes, and a net decanalizing mutation can never invade. Whether a canalizing mutation can invade in this situation depends on whether it is able to reduce the variation caused by other genes enough to compensate for the fitness reduction caused by its own effect on the trait. Hence, in a stable environment, canalization can increase as long as mutations that canalize enough to overcome the conservative force are possible. As the canalizing force gets less effective with increasing canalization and with reduced variation, this balance shifts towards the conservative force as canalization increases and as stabilizing

selection gets stronger. This explains why canalization is harder to achieve with strong stabilizing selection (Wagner et al. 1997, Hermisson et al. 2003; Álvarez-Castro et al. 2009; Fierst and Hansen 2010; and simulations in this paper). Nevertheless, the evolution of complete canalization is a theoretical possibility. In a bilinear system simultaneous canalization of all loci occurs precisely when the vector of reference effects is $\mathbf{g} = -\mathbf{E}^{-1}\mathbf{1}$, where $\mathbf{E} = [{}^{ij}\varepsilon]$ is the matrix of epistasis coefficients, and $\mathbf{1}$ is a vector of ones (Hermisson et al. 2003). In this case the expected phenotype is $z = z_r - (1/2)\ \mathbf{1}^{\mathrm{T}}\mathbf{E}^{-1}\mathbf{1}$, where $\mathbf{1}^{\mathrm{T}}\mathbf{E}^{-1}\mathbf{1}$ is the sum of the elements of $\mathbf{E}^{-1}$. Hence, under the constraint of keeping the trait at the optimum, complete canalization is only possible under the strict requirement that the sum of the elements of $\mathbf{E}^{-1}$ equals twice the difference between the optimum and the reference phenotype (note that this sum must then be zero if $\mathbf{E}$ is measured in a reference with the trait at the optimum). Hence, complete canalization will usually be selected against because it conflicts with optimization of the trait mean. It is also unlikely that minimum canalization consistent with trait optimization can be achieved, because the canalizing force weakens with increasing canalization and therefore gets less and less efficient in overcoming the conservative selective force or even genetic drift and mutation bias away from a lower boundary (Fig. 1).

In a fluctuating environment, the system additionally becomes influenced by ongoing adaptation of the trait mean, which induces correlated responses in canalization. The directions of those correlated responses depend on whether the (de)canalizing alleles happen to increase or decrease the trait value, and will thus alternate as the trait shifts around the optimum. There is no inherent asymmetry in this process, but the system will shift away from any state of strong canalization where there are few possibilities for further canalization and many for decanalization. Thus, fluctuations do not favor decanalization per se, because the canalizing force does not disappear, but the addition of alternating indirect selection should shift the system towards equilibria that are less canalized than expected under pure stabilizing selection. Under some circumstances a fluctuating environment may also favor adaptive decanalization by temporarily shifting the population into convex parts of the fitness landscape (i.e. by making $s < 0$). It is also possible that fluctuations could interact with asymmetries of the genotype–phenotype map such as directional epistasis to produce different outcomes.

Since the long-term multiplicative fitness function in a fluctuating environment is comparable to a constant stabilizing selection function, it can be conjectured that the decanalizing effect is most effective when the period of the fluctuations is long enough to allow substantial allele-frequency change within each period of directional selection.

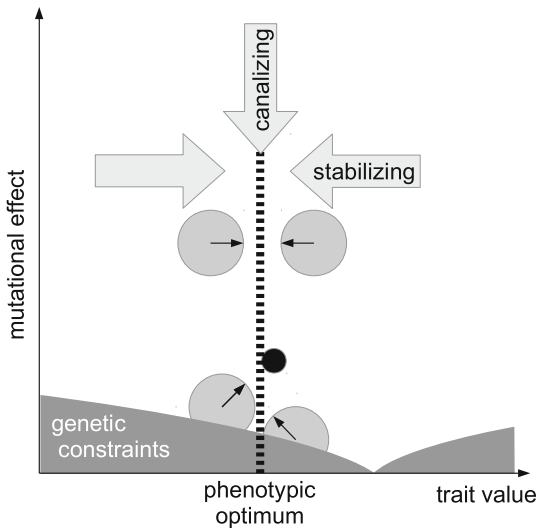

Fig. 1 Illustration of factors influencing canalization. *Whole* and *broken discs* represent the mutational space around a genotype. The area labeled "genetic constraints" is not reachable due to the structure of the genotype–phenotype map. Only for certain trait values can complete canalization be achieved. Stabilizing selection (*big arrows*) pushes the trait towards the optimum, but also generates canalizing selection to reduce mutational effects. If the system is close to maximal canalization, any selection to bring the trait towards the mean will generate correlated responses in mutational effects that are biased towards decanalization (*black arrows* within mutational space). The balance of these forces results in a partially canalized equilibrium (*black dot*), which may be shifted slightly off the trait optimum due to conflicts between trait optimization and canalizing selection

Hence, we predict that decanalization will increase with the period of the fluctuations and with the strength of directional selection experienced within a fluctuation.

Evolution of Epistasis

As shown in "Appendix 2", Eqs. 7 and 8 can be expanded to include higher-order epistatic interactions. Higher-order interactions involving the locus x lead to additional terms describing the fitness effects of the x-locus modifying the interactions among other loci. For example, the fitness effect of modifying pairwise epistasis, as measured in the mean reference, is $-s\Sigma_i\Sigma_j\ {}^{ijx}\varepsilon\ ({}^{ijx}\varepsilon + {}^{ij}\varepsilon)\ {}^{i}v\ {}^{j}v$ with sum taken over all pairs of loci i and j (see Eq. 28). The coefficients of this series, ${}^{ijx}\varepsilon\ ({}^{ijx}\varepsilon + {}^{ij}\varepsilon)$, are less than zero precisely when the substitution $x = 0 \rightarrow x = 1$ brings the corresponding pairwise epistasis term $({}^{ij}\varepsilon + {}^{ijx}\varepsilon x)\ {}^{i}y\ {}^{j}y$ closer to zero without changing its sign. Hence, there is a component of canalizing selection that favors the reduction of pairwise epistasis as measured in the mean reference. Consideration of higher-order terms given in "Appendix 2" shows a similar tendency to reduce higher-order epistasis, and we conclude that stabilizing selection generates a similar canalizing force on both additive and epistatic

effects of all orders. This "canalization of epistasis" will also become less efficient with increasing canalization and should reach a balance with other factors before epistasis disappears. We can thus predict that more decanalized systems will also tend to have elevated epistatic effects.

In the absence of higher-order epistasis, the pairwise epistasis coefficients, ${}^{ij}\varepsilon$ do not evolve, but changing reference effects means that the epistasis terms, ${}^{ij}\varepsilon\ {}^{i}y\ {}^{j}y$, will change, and if we measure epistasis with reference to the state of an evolving population this will manifest itself as changes in the measured epistasis coefficients. Hermisson et al. (2003) showed that under stabilizing selection, an evolving reference genotype tends to adjust the architecture to reduce directional epistasis. Our results above add to this by showing that not just directionality, but all "evolvable" epistasis will tend to be reduced under stabilizing selection. In contrast, under directional selection on bilinear architectures, there is a tendency to evolve negative epistasis relative to the direction of selection; this will eventually start to canalize gene effects and simultaneously produce strong epistasis that maintains a large hidden evolvability that can sometimes be released by genetic rearrangement (Hansen et al. 2006). This may be counteracted by positive third-order epistasis, which can decanalize pairwise epistasis in the same way as positive pairwise epistasis will decanalize single-locus effects under directional selection (Hansen et al. 2006).

Neutral Expectations

For comparison, we also briefly outline the evolution of the multilinear system in the presence of genetic drift and mutation alone. Hence, we extend the neutral model of Lynch (1990, 1993) to include multilinear epistasis. In the absence of selection, the reference effects of each locus evolve independently and in accordance with Lynch's model. Hence, the change in a mean locus reference effect, ${}^{i}\bar{y}$, from generation to generation is normally distributed with mean zero and variance equal to ${}^{i}v/N_e$, where ${}^{i}v$ is the genetic variance at the locus, and N_e is effective population size (Lande 1976). In a balance between drift and mutation, the equilibrium variance will be ${}^{i}v = 2N_e\ {}^{i}v_m$, where ${}^{i}v_m$ is the amount of new variance in allelic reference effects that arise by mutation per generation, and hence, the variance in the change of mean reference effect from generation to generation is $2\ {}^{i}v_m$ (Lynch 1990; Lynch and Hill 1986). After t generations, the mean reference effects are thus independent, normally-distributed variables with mean zero and variances $2\ {}^{i}v_m\ t$. From this we can show that the change in the mean phenotype in the multilinear model will have mean zero and variance

$$\begin{aligned}\mathrm{Var}[\bar{z}] &= 2\big(\Sigma_i {}^{i}v_m + (t/2)\Sigma_i\Sigma_j {}^{ij}\varepsilon^2\ {}^{i}v_m\ {}^{j}v_m \\ &\quad + \big(t^2/4\big)\Sigma_i\Sigma_j\Sigma_k {}^{ijk}\varepsilon^2\ {}^{i}v_m\ {}^{j}v_m\ {}^{k}v_m + \cdots\big)t \\ &= 2V_{mr}\big(1 + (t/8)\,{}^{*}\varepsilon^2 V_{mr}^2 + \cdots\big)t,\end{aligned} \tag{9}$$

where $V_{mr} = \Sigma_i^i\ v_m$ is the total mutational variance arising per generation in the reference genotype and ${}^{*}\varepsilon^2 = 4\Sigma_i\Sigma_j\ {}^{ij}\varepsilon^2\ {}^{i}v_m\ {}^{j}v_m/V_{mr}^2$ is a variance-weighted average squared epistasis coefficient (Carter et al. 2005). This then generalizes Lynch's result that $\mathrm{Var}[\bar{z}] = 2V_m t$ in an additive model (see also Fierst and Hansen 2010 for a similar result for quasi-neutral systems drift under stabilizing selection). The effect of epistasis on the neutral model of phenotypic evolution is thus to accelerate the rate of evolution with time. This is caused by a general tendency for decanalization under random change (Hansen and Wagner 2001a; Hermisson and Wagner 2004). We can see this from the evolution of the epistasis factors. Under bilinear epistasis, the epistasis factors after t generations of neutral evolution are normally distributed with mean one and variance

$$\mathrm{Var}\left[{}^{g\to i}f\right] = 4\Sigma_j {}^{ij}\varepsilon^{2\,j}v_m t. \tag{10}$$

Although there is no bias in favor of positive or negative changes, the mean absolute value and hence decanalization is increasing simply because canalization has a lower limit at ${}^{g\to i}f = 0$. This means that the effects of new mutations will keep increasing under neutral evolution, and this will happen at a rate that is determined by a weighted sum of the squared epistasis coefficients.

Genetic Variance Maintained in Mutation-Selection Balance

The maintenance of genetic variation in the multilinear model by a balance between mutation and stabilizing selection has been studied in detail by Hermisson et al. (2003) and Álvarez-Castro et al. (2009). To understand mutation-selection balance with epistasis, it is important to realize that there are two levels of evolution. The first is the establishment of equilibrium genetic variance with a given genetic architecture, and the second is alteration of that equilibrium through the evolution of the genetic architecture itself. With a fixed architecture the main finding is that any form of multilinear epistasis leads to a reduction of the level of additive, and under most conditions also total, genetic variance maintained in the system relative to an additive model with the same parameter values (Hermisson et al. 2003).

An evolving genetic architecture complicates the dynamics by making the mutation-selection equilibrium itself an evolving entity. Due to the general canalizing tendency of stabilizing selection, this may reduce the genetic variance even further by reducing the effects of individual allele

substitutions, but this effect is counteracted by increasing accumulation of "molecular" variation (${}^{i}v$) under increasing canalization. This dynamics is different in the Gaussian and House-of-cards regimes of mutation-selection balance, and we discuss them in order.

The Gaussian regime is an approximation based on high mutation rates and low mutational effects (see Turelli 1984 or Hermisson et al. 2003 for details), and applies to our simulations below. Hermisson et al. (2003) showed that the equilibrium locus variances of the multilinear map in the Gaussian regime are

$$ {}^{i}v = \sqrt{\frac{{}^{i}v_m}{E[{}^{g\to i}f^2]s}}, \tag{11}$$

where ${}^{i}v_m$ is the mutational variance that arises per locus, i, per generation measured in the reference genotype, and the expectation is taken over the genotypes, g, segregating in the population. Increasing canalization in the current population relative to the reference genotype means per definition that the ${}^{g\to i}f^2$ are less than unity. Hence, the equilibrium variances measured relative to a fixed reference genotype are increasing with increased canalization. This describes the accumulation of "molecular" variation due to weakened selection on alleles with canalized effects. This variation is, however, "hidden" in the current population. An approximation to the realized variation can be obtained by measuring the parameters relative to the evolving average genotype, $\bar{g}$, and under the Gaussian regime this is in fact decreasing with canalization. This is because the mutational variance is decreasing when measured with reference to a more canalized population average; formally, if ${}^{i}v_{mr}$ is the mutational variance measured in a fixed reference, r, then the mutational variance measured in the average genotype is ${}^{i}v_m = {}^{\bar{g}\to i}f_r^2\,{}^{i}v_{mr}$, where the epistasis factor measures canalization of $\bar{g}$ relative to r. One could think that the epistasis factor in the denominator of Eq. 11 would balance this, but since the average epistasis factor is unity when measured in the population mean, we get $\mathrm{E}[{}^{g\to i}f^2] = 1 + \mathrm{Var}[{}^{g\to i}f]$, which may decrease with canalization, but only towards a lower boundary of one. Hence, we are left with an approximately linear decrease of the standing locus variances, ${}^{i}v$, with canalization (measured by ${}^{\bar{g}\to i}f_r$ relative to a fixed reference, r), which also implies an approximately linear decrease of the canalizing force with increasing canalization (seen by substituting Eq. 11 into Eq. 8).

Several predictions can be drawn from this. First, Hermisson et al. (2003) showed that the equilibrium additive variance in the Gaussian regime is given by sum of the ${}^{i}v$ measured in the mean reference as

$$V_A = \Sigma_i {}^{i}v = \Sigma_i \sqrt{\frac{{}^{i}v_m}{(1 + Var[{}^{g\to i}f])s}}, \tag{12}$$

where the variance is taken over all genotypes, g, segregating in the population, and all parameters are measured relative to the average genotype, $\bar{g}$. Hence, because
the mutational variances decrease with the square of the canalization (the epistasis factor), we predict that the additive variance will decrease approximately linearly with canalization (at least when canalization is strong, so that the variance in epistasis factors can be ignored). The second prediction is that there should be an approximate equilibrium level of non-zero canalization in mutation-selection balance. This follows because the ${}^{i}v$ and therefore the strength of the canalizing force in Eq. 8, decreases linearly with canalization. Third, since the canalizing force here scales with the square root of s, while the conservative (and the adaptive) forces scale with s, there will have to be a point were equilibrium canalization starts to decrease with increasing strength of selection.

In the House-of-cards regime, which applies to rare mutations with large effects, the equilibrium variance depends only on the mutation rate and not on the mutational effects (Turelli 1984). For the multilinear model, the equilibrium variances are

$$ {}^{i}v = \frac{{}^{i}u}{E[{}^{g\to i}f^2]s}, \tag{13}$$

where ${}^{i}u$ is the rate at which new mutations appear per locus per generation (Hermisson et al. 2003). Here, the standing molecular variance measured in the mean reference is not decreasing with canalization. In fact there will be a decelerating increase due to the factor $\mathrm{E}[{}^{g\to i}f^2] = 1 + \mathrm{Var}[{}^{g\to i}f]$ in the denominator. Hence, in the house-of-cards regime, the canalizing force is not weakening, but slightly strengthening, with increasing canalization, and the prospects for evolving very strong canalization are better. We still do not expect maximal canalization, however, because the availability of canalizing mutations will decrease as discussed above, and reduced mutational effects may eventually also shift the system into the Gaussian regime. We can also note that the canalizing force is independent of the strength of stabilizing selection (s) in the House-of cards model (as in the Haldane–Muller principle), and this should give an earlier and more pronounced decanalization with increasing selection strength than in the Gaussian regime.

These remarks pertain to equilibria under constant stabilizing selection, but due to the similarity of the multiplicative fitness functions, we predict similar scaling relationships of standing variances in short-term fluctuating environments. The main prediction is that equilibrium additive variance will scale approximately linearly with (strong) canalization in the Gaussian regime (but there is no such prediction under the House-of-cards regime). This

conjecture is complicated by the direct effects of fluctuations on the maintenance of variance, which are sensitive to types of fluctuations and allelic effect sizes (see discussion), and we turn to numerical experiments to test our conjectures.

Numerical Experiments

Methods

The numerical experiments are based on the individual-based simulation program described in Hansen et al. (2006) with slight modifications. The model considers evolution in a population of constant size $N = 1{,}000$ diploid individuals. The genotypic value of each individual is determined by a bilinear genotype–phenotype map (Eq. 3 ignoring higher-order terms) with 20 loci, and the reference effect of each locus is given as the sum of the two allelic reference values assigned to the individual. To this is added a random environmental effect drawn from a normal distribution with mean zero and unit variance, which hence sets a scale for the other parameters. The genotypic value of the reference genotype is set to $z_r = 0$. Before reproduction each individual is assigned a fitness based on its phenotypic value. To make an offspring for the next generation, two parents are then drawn at random with replacement from the population with a probability proportional to their relative fitness. For each locus each parent contributes one allele drawn with equal probabilities from the two alleles of the parent. As previous studies have shown that only tight linkage are likely to have significant effects on the dynamics of the model (Carter et al. 2005), we assume free recombination by drawing alleles at different loci independently.

Genetic variation is generated by mutation of the allelic reference effects. This happens with probability 0.01 per allele per generation, and the change in value is drawn from a normal distribution with mean zero and standard deviation, 0.025 (i.e. 2.5 % of the environmental standard deviation). The unrealistically high rate of mutation per allele was chosen to speed up simulations. Preliminary explorations and results from Hansen et al. (2006) and Fierst and Hansen (2010) indicate that elevated mutation rates are not likely to have qualitative effects on the results beyond a change of time scale. Note also that the total amount of new mutational variance initially added per generation, $V_m = 0.01V_e$, is high, but not outside empirical bounds (Lynch 1988; Houle et al. 1996; Houle 1998).

For each simulation, the whole set of 190 epistasis coefficients, ${}^{ij}\varepsilon$, for 20 loci were drawn independently from a normal distribution with a specified mean and variance. A mean different from zero gives directional epistasis. To compare directional and nondirectional architectures on an equal footing we set the parameter values to make the average absolute value of the epistasis coefficients equal (see Fierst and Hansen 2010 for details). Epistasis coefficients have units inverse to the units of the trait. To interpret their size we must relate them to the effects of the allele substitutions they modify. For example a mutation of size ${}^{i}m = 0.025$ at locus i will modify the effect of a substitution at locus j with a factor ${}^{i\to j}f = 1 + {}^{ij}\varepsilon {}^{i}m$, which is a 2.5 % change when ${}^{ij}\varepsilon = 1$. The strongest epistasis in our simulations is of this magnitude. On a locus-by-locus basis this is weak and hard-to-detect epistasis, but since we allow all 20 loci to affect each other it still adds up to a substantial potential for evolution of gene effects.

For the majority of simulations we used a Gaussian fitness function $\mathrm{W}(z) = \mathrm{Exp}[-s\,(z-\theta)^2]$, in which the optimum, θ, was allowed to change according to different rules. We focused on simulations in which θ was drawn anew every T generations from a normal distribution with variance σ^2_θ. To explore the effects of convexity we explored a reflected exponential ($\mathrm{W}(z) = \mathrm{Exp}[-s|z-\theta|]$) and a quadratic ($\mathrm{W}(z) = 1 - s(z-\theta)^2$) fitness function (note that the parameter s does not mean exactly the same in these functions. We chose the forms so that the reduction in fitness are approximately the same when $|z-\theta| = \sigma_e = 1$). We also explored non-stationary evolution of θ according to a Brownian-motion process with different rates, and three types of fluctuating directional selection, in which the parameter s was allowed to fluctuate in the fitness functions $\mathrm{W}(z) = 1 + s(z - \mathrm{E}[z])$, $\mathrm{W}(z) = \mathrm{Exp}[s(z - \mathrm{E}[z])]$, and $(1/2)\,\mathrm{Log}[1 + 2s(z - \mathrm{E}[z])]$. Any negative fitness values were set to zero.

We monitored the outcome of the simulations by recording changes in average mutational effects and standing additive variance, computed as $V_A = \Sigma_i\, {}^{i}v$, with ${}^{i}v$ measured in the mean reference. Hence, we ignore any effects of deviance from Hardy–Weinberg and linkage equilibrium.

Results

In a purely additive genetic architecture, a balance between mutation and Gaussian stabilizing selection is reached within a few thousand generations with our parameter values (Fig. 2), and with a fluctuating optimum a stochastic equilibrium is reached in a similar amount of time (Fig. 3a). The effects of period and amplitude of fluctuations are illustrated in the contour diagram in Fig. 3b. As predicted from theory, fluctuations over one or a few generations have little or no effect on the average additive variance with a Gaussian fitness function, but when the period reaches about ten generations or more and shifts are of large amplitude, we start to see reduced levels of additive variance. This effect gets stronger when the period

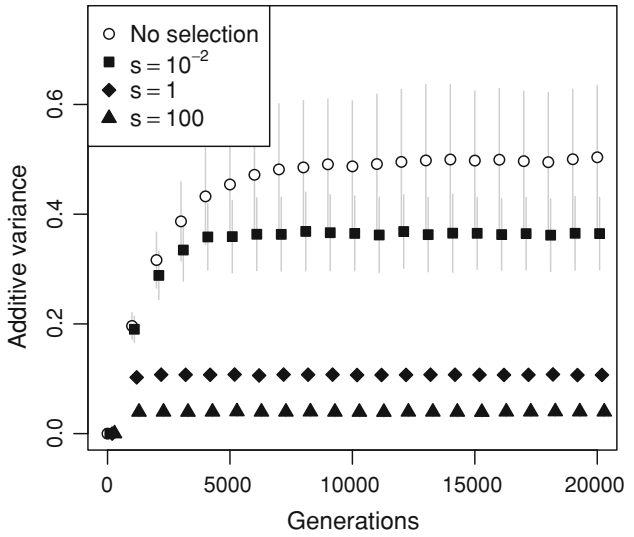

Fig. 2 Additive genetic architecture under Gaussian stabilizing selection: The time development of the additive variance in units of environmental variance averaged over 1,000 repetitions is shown for a Gaussian fitness function acting on 20 additive loci that experience mutations with rate 10^{-2} per allele per generation and effects drawn from a normal distribution with standard deviation 0.025 of the environmental standard deviation in a population of N = 1,000. Four different strengths of selection are shown including a case of neutral evolution. The *bars* cover one standard deviations in each direction over the repetitions

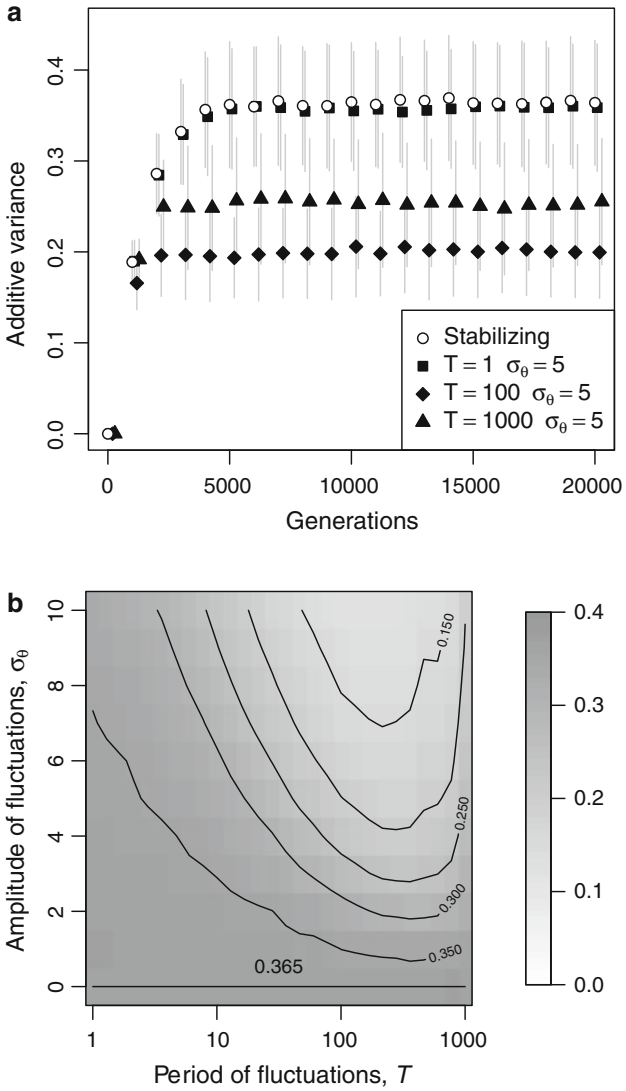

Fig. 3 Additive genetic architecture under a Gaussian fitness function with a fluctuating optimum, and strength of selection, $s = 0.01$. **a** Time plot of standing additive genetic variance for selected periods of fluctuation, T, in units of generations, and standard deviation of amplitudes, σ_θ, in units of environmental standard deviations. **b** Contour plot of the additive variance maintained on average between ten- and fifty-thousand generations (based on 280 grid points each averaged over 100 repetitions for each parameter set; each repetition averaged over data taken every thousand generation). The *bottom line* represents stabilizing selection (constant optimum). Other settings as in Fig. 2

increases up to several hundred generations, but then weakens on time scales where populations reach equilibria within long periods of constant stabilizing selection. Hence, we found no indication that a fluctuating optimum in a Gaussian fitness landscape can elevate the evolvability of a polygenic additive architecture. Instead, we found moderately reduced additive variance under large-amplitude fluctuations with "intermediate" periodicity of about ten to thousand generations.

Evolution of canalization is not possible with an additive architecture. We now consider the effects of epistasis. We first verify, in Fig. 4, the prediction of an inherent, accelerating trend towards decanalization for a neutral trait with an associated continued increase in additive genetic variance. As predicted there is no difference between directional and non-directional epistasis. Introducing Gaussian stabilizing selection in Fig. 5, we observe the evolution of canalization in the form of reduced mutational effects. This appears a little faster with directional than with non-directional epistasis, but the two are qualitatively similar. In all cases, canalization appears to reach a non-zero (stochastic) equilibrium, and, as illustrated in Fig. 6, intermediate strengths of stabilizing selection lead to more canalized equilibria. The additive variance is in all cases reduced relative to the comparable additive architectures explored in Fig. 2.

In Fig. 7 we illustrate how fluctuating fitness optima induce decanalization relative to constant stabilizing selection. Although it is not clear that a stochastic equilibrium has been reached in all cases even after fifty-thousand generations, we decided to use averages taken between ten- and fifty-thousand generations as indicators of the effects of period and amplitude of fluctuations. The resulting contour diagrams in Fig. 8 show that canalization decreases with increasing amplitudes of fluctuations, but the effect is small for amplitudes below about five environmental standard deviations. The most pronounced

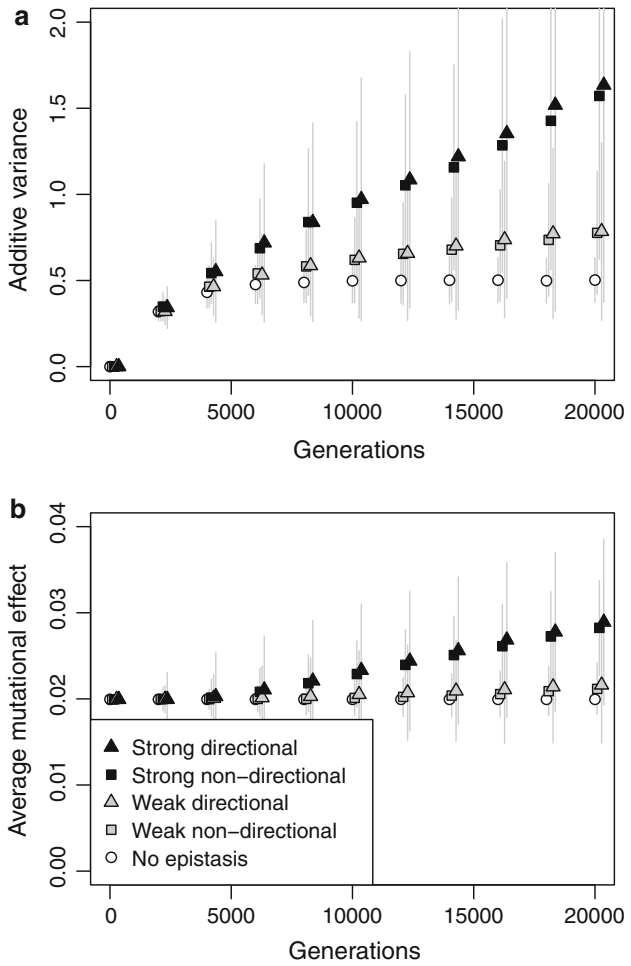

Fig. 4 Epistatic architectures under neutral evolution. **a** Additive variance in units of environmental variance. **b** Average mutational effect (canalization) in units of environmental standard deviations. Epistasis coefficients, ε, for interactions between pairs of 20 loci are drawn independently from a normal distribution in each replicate simulation. Five cases are considered: no epistasis $\varepsilon \sim N(0, 0)$, weak non-directional epistasis $\varepsilon \sim N(0, 0.5)$, weak directional epistasis $\varepsilon \sim N(0.25, 0.430)$, strong non-directional epistasis $\varepsilon \sim N(0, 1)$, and strong directional epistasis $\varepsilon \sim N(0.5, 0.859)$. Other settings as in Fig. 2

effects are found for periodicities between about ten and two-hundred generations. As expected, fluctuations on very short or very long periods produce effects more similar to stabilizing selection. As predicted, the equilibrium additive genetic variance tracks the canalization, and for extreme amplitudes (above about 8-10 environmental standard deviations) the additive variance start to exceed the levels found in the purely additive model explored in Fig. 2. Directional and non-directional epistasis have qualitatively similar effects, which may reflect the predicted disappearance of directional epistasis over time.

We have shown that the multiplicative long-term fitness landscape induced by a Gaussian fitness function with a fluctuating optimum will be identical to that of a Gaussian fitness function with a constant optimum. In this light, it is not surprising that we found small effects of fluctuations with short period where there is less scope for the trait mean to shift around. These results may not hold with other shapes of the fitness function, however, and in Fig. 9 we illustrate how a rapidly fluctuating optimum in a more convex fitness function (the reflected exponential) leads to substantial decanalization relative to the Gaussian shape, and also relative to a constant fitness landscape. In contrast, a more concave function (the quadratic) only has a very slight effect, producing slightly stronger canalization than the Gaussian. The effects of convexity are even more dramatic with fluctuating directional selection (Fig. 10). Here, a convex form (the exponential) leads to much more decanalization than a linear form, which again is more decanalized than a concave (log) form. Note that all these results hold with very short periods of fluctuation (one generation).

Finally, we explored the effects of nonstationary changes in the environment where the optimum can move far from the starting point. We considered three different models, a Gaussian optimum changing as a Brownian motion, a fluctuating linear fitness function (where the population mean will drift like Brownian motion), and a single shift in a Gaussian optimum. In Fig. 11 the results are plotted against the position of optimum (or population mean in case of fluctuating linear selection) after 20,000 generations. This shows that less-canalized equilibria are associated with larger shifts in the mean. As expected there is a strong effect of directionality of epistasis with pronounced decanalization when the trait has shifted in directions of positive epistasis. When the trait has shifted in directions of negative epistasis, the situation is more complicated. Small shifts are associated with canalization (relative to stabilizing selection), while larger shifts are associated with decanalization relative to stabilizing selection, although not as much as under non-directional epistasis. The shift to decanalization after large changes under negative epistasis likely involves the evolution of negative epistasis factors relative to the starting architecture (see Hansen et al. 2006 for detailed discussion). Again, levels of standing additive variance closely track the canalization, and increased evolvability is predicted for populations that have experienced a substantial shift in trait mean in their recent history.

Discussion

Selection in a Fluctuating Environment

The temporal multiplication of fitness in a fluctuating environment tends to favor types that minimize their

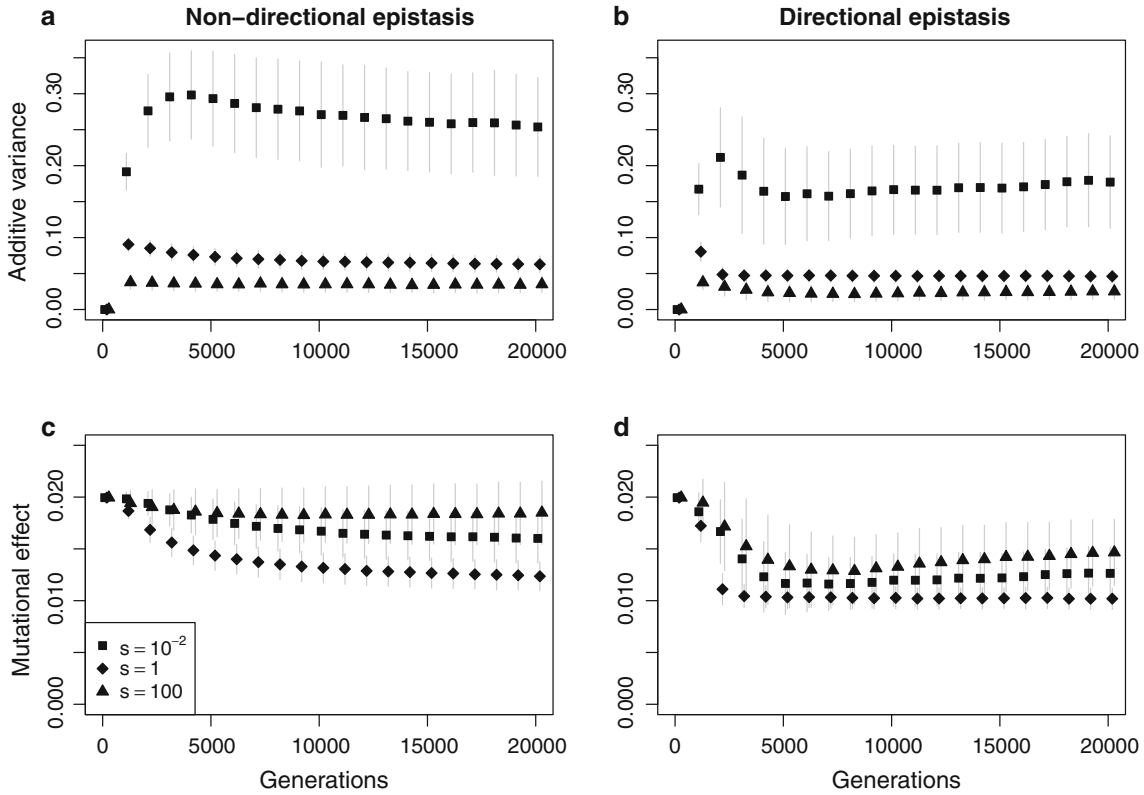

Fig. 5 Epistatic architectures under stabilizing selection: The plots show the time development of additive genetic variance (**a**, **b**) and average mutational effect (**c**, **d**) for **a**, **c** non-directional epistasis ($\varepsilon \sim N(0, 1)$), and **b**, **d** directional epistasis ($\varepsilon \sim N(0.5, 0.859)$). In each panel three strengths of Gaussian stabilizing selection are shown. Other settings as in Fig. 4

temporal variance in fitness (e.g. Levins 1968; Frank and Slatkin 1990; Lande 2007, 2008). Intermediate or generalist phenotypes that do well in all circumstances tend to have the highest multiplicative fitness. This may lead to protected polymorphisms through superiority of the heterozygote phenotypic intermediates (Haldane and Jayakar 1963; Gillespie 1973, 1991; Turelli 1981), it may favor bet-hedging strategies (Bull 1987), and it may generate stabilizing or normalizing selection on phenotypes. The criterion for long-term multiplicative selection to be stabilizing is related to log concavity of the fitness function. Hence, multiplicative selection may become long-term stabilizing selection even if there is no concavity, or even weak convexity, of observed within-generation fitness functions. The surprisingly common observations of convex fitness functions in field studies (Kingsolver et al. 2001; Blows and Brooks 2003; Stinchcombe et al. 2008) may therefore be consistent with long-term stabilizing selection.

All these outcomes can be viewed as a result of selection against variation and for robustness in response to environmental fluctuations. From this perspective it is surprising that fluctuating selection is often judged to be decanalizing and favorable to evolvability (e.g. Wagner 1996; Flatt 2005; Lee and Gelembiuk 2008). Evolvability and robustness are, however, not necessarily in conflict (e.g. Wagner 2005, 2008). Robust or canalized systems preserve molecular variation and this hidden variation may be released during an evolutionary change (Hermisson and Wagner 2004), protected polymorphisms preserve alleles that may be used to fuel subsequent directional selection, and increased systems drift in a robust system may expose evolutionary novelties (Wagner 2005). Furthermore, the effects of fluctuating selection are not reducible to its long-term average, and there are several temporal effects that can boost evolvability. Fluctuations of longer periods may elevate short-term evolvability by shifting rare alleles to higher frequencies (see below), and we have shown here that the shifting trait mean generates indirect selection that may counteract direct canalizing selection. Predictable fluctuations may also select for phenotypic plasticity (e.g., Lande 2009), which stands in a complex relationship to genetic evolvability that can be favorable or disfavorable (Paenke et al. 2007).

Evolution of Canalization

Indeed, the simulations presented here, as well as those of Kawecki (2000), show that may types of fluctuating fitness

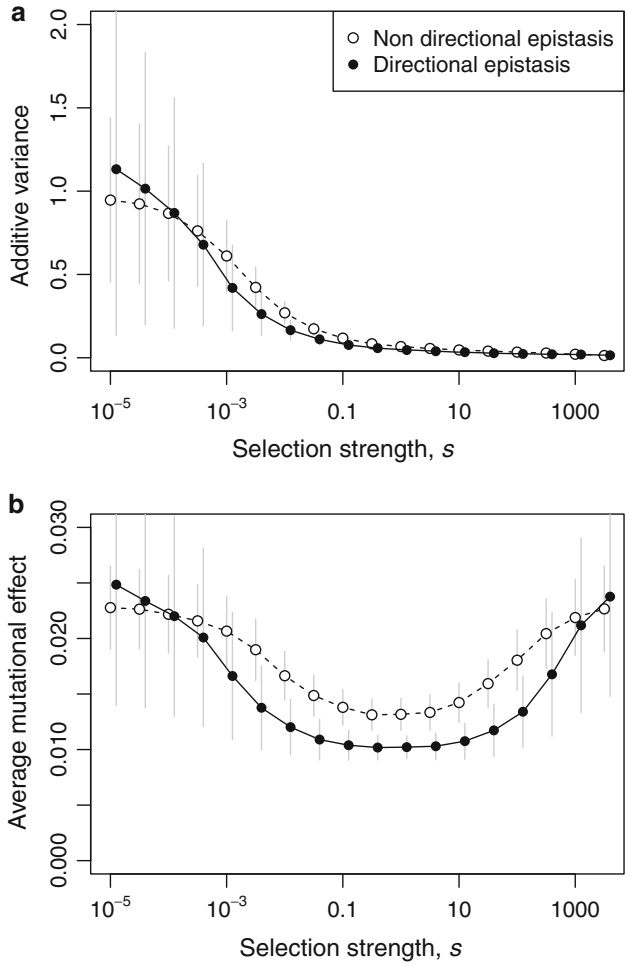

Fig. 6 Equilibrium canalization in relation to strength of stabilizing selection. Each point shows the average mutational effect after 10,000 generations averaged over 1,000 repetitions for the same directional and non-directional epistatic architectures as in Fig. 5. Other settings as in Fig. 5

optima make epistatic systems evolve to become more decanalized and more evolvable than comparable systems under pure stabilizing selection. This is not because fluctuating environments directly select for decanalization. Indeed, traits under stationary fluctuations experience the same canalizing selection as we find in traits under pure stabilizing selection, and are more canalized than what we would expect under neutral evolution, or under directional selection on positive epistatic architectures. We propose the reason why fluctuating selection leads to less canalized systems than stabilizing selection is that canalization becomes influenced by indirect selection due to adaptation of the trait mean. The back and forth movements of the trait mean produce correlated responses in canalization that swamp the canalizing selection resulting from the concavity of the multiplicative fitness function and preclude the system from evolving as strong canalization as it might under constant stabilizing selection. Our simulations show that moderate decanalization can occur under fluctuating selection of large amplitude, and that this may elevate evolvability even in the face of a general tendency for both epistasis and fluctuations to reduce additive variance.

Our analysis shows that the effects of quadratic trait selection on a potentially canalizing locus can be decomposed into (1) a component due to adaptation of the trait mean, (2) canalizing selection caused by a fitness load due to genetic variation in the loci that are the targets of canalization, and (3) conservative selection against rare alleles caused by the on-average disfavoring of change in a concave fitness landscape. The conservative force is the least obvious, and although it does not act directly on canalization, it is instrumental for predicting its dynamics. The finding that the rate of canalization increases with the strength of stabilizing selection only up to a point for then to decrease (Fig. 5; Wagner et al. 1997; Fierst and Hansen 2010) can be understood as result of the conservative force increasing more rapidly than the canalizing force with strength of selection.

Canalization and Genetic Architecture

For canalization to evolve, gene effects must be evolvable. This requires that effects of allele substitutions are dependent on the state of the rest of the genome, and can thus not happen in a purely additive model. While gene effects could evolve due to interactions between allelic states and subsequent mutations at the same locus (Hansen 2006, 2011), their evolution is usually thought to involve epistasis between loci. Most investigations of canalization have simplified this by imagining a hypothetical modifier locus that alters the effects of other genes without itself having an effect on the trait. Although a useful theoretical tool to identify canalizing selection, it is important to realize that pure modifiers can not exist. Epistasis is symmetrical and it can be proven formally that any gene that modifies the effect of another gene on a trait must also itself have effects on that trait (Hansen 2011). This implies that the evolution of gene effects, canalization, epistasis, and evolvability are all affected by correlated responses to trait evolution. Because direct selection caused by canalization, as in our canalizing force, is often weak (e.g. Proulx and Phillips 2005), it follows that canalization becomes dominated by indirect selection when the trait mean changes due to directional or fluctuating selection. This makes the evolutionary outcome dependent on the genetic details that determine the correlation between canalizing effects and direct trait effects. To a first approximation this correlation is determined by the directionality of epistasis, with positive epistasis in the direction of trait change causing decanalization and negative epistasis causing canalization. This explains the crucial role of

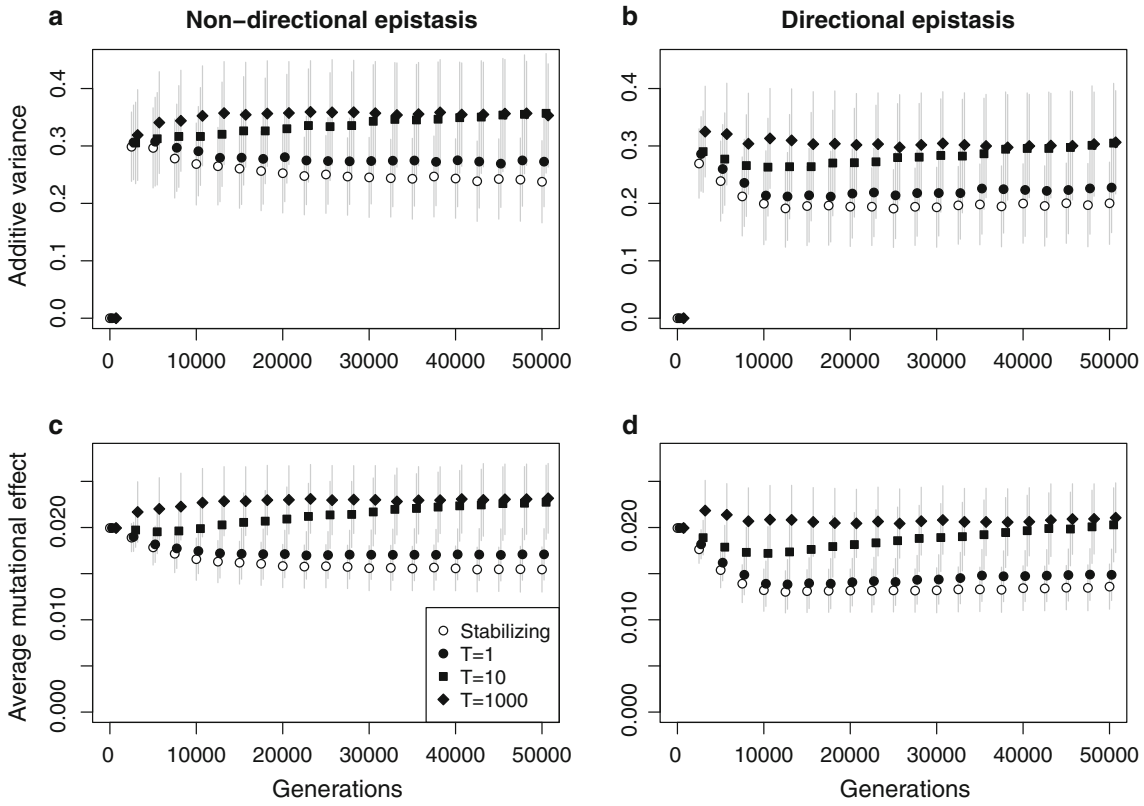

Fig. 7 Epistatic architectures under a Gaussian fitness function with a fluctuating optimum: **a**, **b** Time plots of additive variance. **c**, **d** Time plots of average mutational effect for **a**, **c** non-directional epistasis ($\varepsilon \sim N(0, 1)$) and **b**, **d** directional epistasis ($\varepsilon \sim N(0.5, 0.859)$. In all cases the optimum of a Gaussian fitness function with $s = 0.01$ fluctuates with amplitude $5\sigma_E$ and periods $T = 1$, 10 or 1,000 generations. Constant selection with same parameters is shown for comparison. Other settings as in Fig. 4

directional epistasis in the evolution of evolvability under directional selection (Carter et al. 2005; Hansen et al. 2006; Yukilevich et al. 2008; Pavlicev et al. 2010), as well as its effects under non-stationary fluctuations where the trait mean can shift far from the ancestral value (see Fig. 11).

The importance of indirect selection and genetic details also makes it essential to consider general genetic systems when studying the evolution of evolvability. The highly specific models of the genotype–phenotype map used in many simulation studies may produce model-specific outcomes due to accidental built-in correlations between trait effects and evolvability (Gardner and Zuidema 2003; Hansen 2011). In this study we have used the multilinear epistatic model of the genotype–phenotype map. This model is an extension of the additive model that can represent any form of linear stretching or compression of gene (and genotype) effects. It may be regarded as a first-order approximation for the evolvability of gene effects and canalization. It is, however, quite flexible in its ability to represent genotype–phenotype maps. For example, if there are only two possible genotypic values at each locus or if all alleles within a locus are required to be additive in all genetic backgrounds, then the multilinear model is completely general. Importantly, it allows canalization to stand in both positive and negative relation to trait effects. Nevertheless, in the multilinear model, dominance and overdominance can not evolve on a locus without initially being present, and this limits the opportunity for creating (or removing) hetrosis that may have strong effects on the maintenance of genetic variation. By assuming that alleles act additively within loci, we have therefore limited the scope for evolution of protected polymorphisms. Although this may be less important in a polygenic system, it means our results can not be generalized to cases with major-effect loci. Other epistatic architectures may allow stable internal equilibria that can maintain genetic variation even in the absence of mutation (Gimelfarb 1989; Zhivotovsky and Gavrilets 1992; Gavrilets 1993; Gavrilets and de Jong 1993; Kirzhner et al. 1998; Liberman and Feldman 2005). On the other hand, sign epistasis, where the identity of the fittest locus genotype may change, may also act as a constraint on evolution (Weinreich 2005; Weinreich et al. 2005), and the dynamics becomes sensitive to genetic details. See also Liberman and Feldman (2005, 2006, 2008), Gardner and Kalinka (2006), Desai et al. (2007), and Liberman et al. (2007) for results on evolution of gene

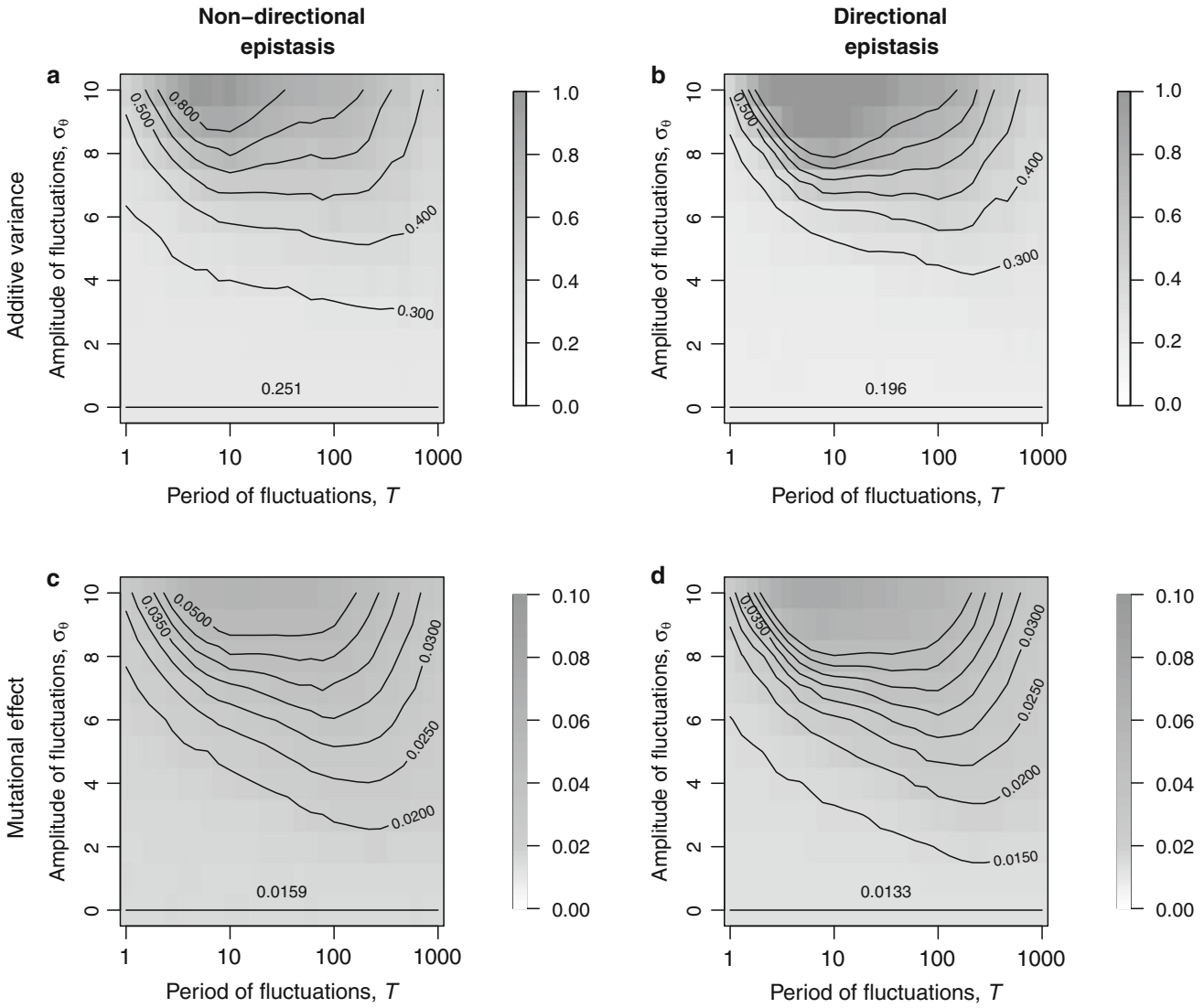

Fig. 8 Effects of period and amplitude of fluctuations on epistatic architectures: Contour plots of **a**, **b** average mutational effect, and **c**, **d** additive variance for **a**, **c** directional epistasis ($\varepsilon \sim N(0.5, 0.859)$) and **b**, **d** non-directional epistasis ($\varepsilon \sim N(0, 1)$). Settings for contour plots as in Fig. 3b. The *bottom line* represents stabilizing selection (constant optimum). Other settings as in Fig. 7

effects and epistasis in specific two- and three-locus systems.

Maintenance of Genetic Variation in a Fluctuating Environment

Despite the importance of environmental fluctuations and a large technical literature on mutation-selection balance, only a handful of studies have combined the two (Bürger 2000). Bürger (1999) and Bürger and Gimelfarb (2002) investigated the effects of fluctuating selection and mutation on the maintenance of genetic variation in a trait determined by an additive genotype–phenotype map. Their analyses were based on a Gaussian fitness function with a moving optimum. From our perspective, their main finding was that fluctuations with short periods ($\leq$8 generations) did not make much difference from pure stabilizing selection, but fluctuations with longer periods ($\geq$24 generations) did elevate segregating genetic variance. This supports earlier findings that short-term fluctuations (white noise) in the optimum of a Gaussian fitness function do not affect the maintenance of genetic variance in the additive model (Felsenstein 1976; Lande 1977; Turelli 1988). The increase in genetic variance under fluctuations with longer periods is related to findings that genetic variance in additive models is increased under constant directional selection relative to levels under stabilizing-selection-mutation balance because favorable alleles are then sweeping through the population (Bürger and Lynch 1995; Bürger 1999; Waxman and Peck 1999; Jones et al. 2004,

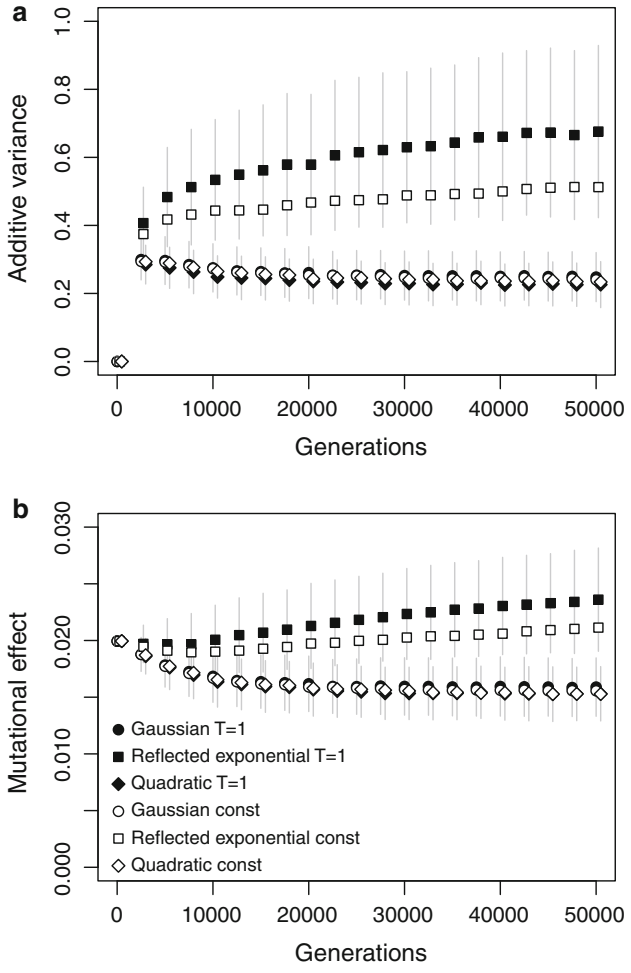

Fig. 9 Effects of convexity in the fitness function: Time developments of **a** additive variance and **b** average mutational effects are shown for a fluctuating optimum ($\theta \sim N(0, 2\sigma_e)$) in quadratic ($W(z) = 1 - s(z - \theta)^2$), reflected exponential ($W(z) = \mathrm{Exp}[-s|z - \theta|]$), and Gaussian ($W(z) = \mathrm{Exp}[-s(z - \theta)^2]$) fitness functions. We show only short-period fluctuations ($T = 1$) to focus on the effects of average convexity. Constant fitness functions ($T = 0$) are shown for comparison. The epistatic architecture is non-directional ($\varepsilon \sim N(0, 1)$), and other settings as in Fig. 7

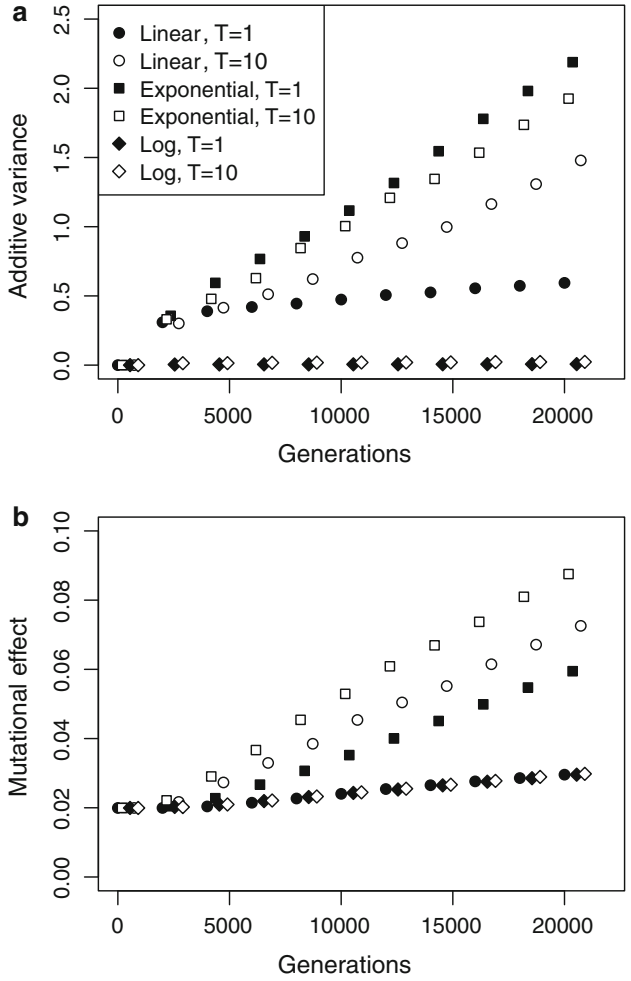

Fig. 10 Effects of fluctuating directional selection on a non-directional epistatic architecture. Time developments of **a** additive variance and **b** average mutational effects are shown for three forms of fluctuating selection. In the linear case, the parameter s in the fitness function $W(z) = 1 + s(z - E[z])$ is drawn as $s \sim N(0, 1)$ every T generations. In the exponential case, the parameter s in the fitness function $W(z) = \mathrm{Exp}[s(z - E[z])]$ is drawn as $s \sim N(0, 1)$ every T generations. In the logarithmic case the parameter s in the fitness function $W(z) = 1 + 1/2(\mathrm{Log}(1 + 2\,s(z - E[z]))$ is drawn as $s \sim N(0, 1)$ every T generation. Two periods, $T = 1$ and 10 generations are shown for each model. Other settings as in Fig. 9

2012). Presumably, fluctuating selection with the right periodicity allows alternating changes in allele frequencies, which increases genetic variance when they reach intermediate values. This makes the results strongly parameter dependent. If initial genetic variation is dominated by a few rare alleles of large effect (the House-of-cards regime) then a rapid increase of variance is expected under directional selection. This explains the orders of magnitude increases of genetic variance under directional selection found by Kondrashov and Yampolsky (1996), because they based their simulations on very strong selection and very low total mutation rates (Bürger 1999). Our simulations are consistent with no effect of short-period fluctuations of Gaussian fitness on additive variance, but we consistently found decreasing and not increasing equilibrium variances under fluctuations with longer periodicity. We attribute this result to the "Gaussian" conditions of high mutation rates and low mutational effects in our simulations, which minimize the transient variance effects of fluctuating allele frequencies. Hence, our simulation results are not transferable to "House-of-cards" conditions with low mutation rate and large mutational effects.

The first empirical studies of the effects of fluctuating selection on the evolution of variational properties in quantitative traits have just appeared. Pélabon et al. (2010) exposed a wing-venation character in two outbred lab populations of

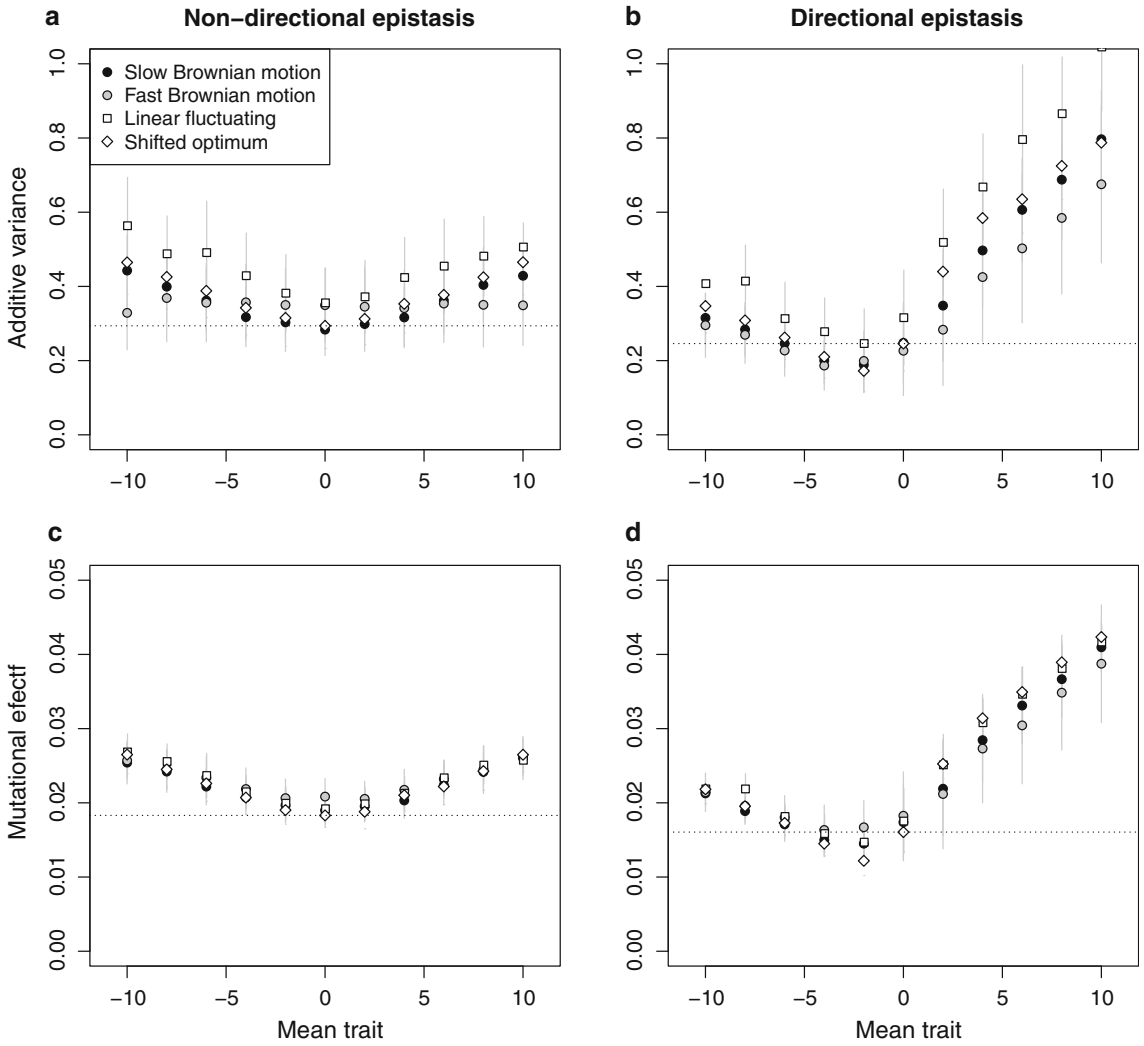

Fig. 11 Effects of non-stationary fluctuations on epistatic architectures: The figures plot additive genetic variance (**a**, **b**) and mutational effects (**c**, **d**) against the deviance of the population mean from the starting point for directional [**b**, **d**, $\varepsilon \sim N(0.5, 0.859)$] and non-directional [**a**, **c**, $\varepsilon \sim N(0, 1)$] epistasis. Three different fluctuating selection regimes are plotted: (1) Gaussian fitness function with an optimum that change according to a Brownian motion with variance equal to 0.01 environmental variance units per generation. (2) Same, but with doubled rate of movement. (3) A fluctuating linear fitness function as in Fig. 10. (4) A Gaussian fitness function with a single shift in the optimum. For Brownian motion and linear fluctuations, 2,000 simulation runs were divided into bins of two phenotypic units based on the end values of, respectively, the optimum and the trait mean. For the single-shift model 100 simulations were conducted with the optimum in each bin. Other settings as in previous figures

Drosophila melanogaster to 20 generations of stabilizing, fluctuating, and disruptive selection treatments. Their main finding was that both stabilizing and fluctuating selection lead to a slight decrease in variation relative to control populations, while there was a large increase under disruptive selection. This is consistent with the theoretical prediction of little difference between stabilizing and fluctuating selection on time scales that are too short for substantial changes in canalization. Decanalization under disruptive selection has also been found in earlier studies (Scharloo et al. 1967, 1972) and is predicted by theory (Rice 1998, 2002; Kopp and Hermisson 2006; this paper), but is unlikely to happen this quickly, and the changes are more likely a result of linkage disequilibrium or increasing frequencies of alleles with extreme effects, which was also evidenced by an increase in the frequency of malformed wings. Le Rouzic et al. (2011) fitted specific genetic models to the same two populations under up and down directional selection, and found some evidence of canalization in the form of negative directional epistasis reducing genetic variance in the up direction, although this yielded to an increase in genetic variance towards the end of the experiment. There were also indications of environmental canalization that may have driven genetic canalization. Hallsson and Björklund (2012a) exposed flour beetles, *Callosobruchus maculatus*, to 18 generations of changing temperature (linear trend, white noise and red noise) and found reduced genetic variation of some

life-history traits in the fluctuating regimes relative to constant trend and control treatments (see also Hallsson and Björklund 2012b). Although these studies show that changes in genetic variance can happen on time scales of tens of generations, these changes are probably dominated by strong non-equilibrium dynamics of the genetic architecture, and the results are not strong tests for the evolution of canalization because systematic differences between stabilizing and fluctuating selection would take much longer time to exert themselves.

Canalization as Inherent Property of Biological Organization

Our finding that a degree of canalization is likely to evolve under any long-term concave stochastically stationary fitness function supports the established view of organisms as canalized (e.g. Waddington 1942, 1957). This is empirically based on the observation that measures of variation or variability often increase under either genetic or environmental perturbance (reviewed in Rendel 1967; Scharloo 1991; Moreno 1994; Hoffmann and Parsons 1997; Rutherford 2000; Gibson and Wagner 2000; Dworkin 2005; Flatt 2005). This view has been challenged by Hermisson and Wagner (2004) based on the finding that random changes to any system is biased towards decanalization (see also Wagner and Mezey 2000; Hansen and Wagner 2001a; Barton and Turelli 2004; Zhang 2008; Zhang and Hill 2010 for related results), and decanalization under perturbations is therefore not necessarily evidence for adaptive canalization of the wild type. Indeed, our finding of accelerating decanalization under genetic drift is a manifestation of this bias. These results show that there is no meaningful null expectation for canalization. We suggest that any system with finite variability is "canalized", and canalization is an integrated part of any recognizably organized biological character. Canalization can therefore only be studied meaningfully in a comparative manner. Our theoretical results predict that systematic differences in canalization may exist depending on the strength and pattern of selection, but as the outcome may be constrained by genetic architecture (e.g. by absence of epistasis or a limited range of allelic reference effects), it may be a modest signal with much residual variation. We also note that genetic canalization is likely subject to two other sources of indirect selection not modeled here, namely selection for speed and accuracy of the general developmental system (Waddington 1957; Hansen 2011) and selection for environmental canalization (Wagner et al. 1997; de Visser et al. 2003).

Canalization, Epistatic Constraints, and Stasis

Our analysis provides little support for the hypothesis that long-term phenotypic stasis is caused by canalization or epistatic constraints. While we have shown that both stabilizing selection and stationary fluctuating selection will generate a canalizing selection pressure on both gene effects and epistatic effects, and that this in theory could produce a completely canalized genetic system void of short-term evolvability, we have also shown that this force is weakening with increasing canalization and likely to be checked or balanced by other forces. There is a tendency for potentially constraining directional epistasis to disappear and no particular tendency for multilinear epistasis to evolve into patterns that would create strong constraints on evolution. Note however, that these results pertain to symmetric fluctuations and we can not rule out the possibility of epistatic constraints for traits under persistent directional selection (Hansen et al. 2006).

Evolution of Evolvability

Evolvability as a quantitative trait may be defined as the ability to respond to a selective challenge. This may be measured on many different levels. In the short term evolvability is determined by the standing additive variance of the character (Houle 1992; Hansen et al. 2011), and in the longer term it is determined by the ability of the genetic system to produce and maintain potentially adaptive variation (Wagner and Altenberg 1996; Hansen 2006). Our results add to a large literature showing that evolvability is evolvable on both these levels (reviewed in Pigliucci 2008), but as to the question of whether variational properties and genetic systems are likely to evolve as adaptations to facilitate evolvability, our results give a negative answer. Although evolvability may be favorable for a population in a fluctuating environment and we have shown that epistatic systems tend to evolve to less canalized states in the presence of a fluctuating fitness optimum, they are still more canalized than expected under neutrality or weaker stabilizing selection, and standing evolvability is typically less than in comparable additive systems unless fluctuations are of very large amplitude (and intermediate periodicity). Importantly, elevation of evolvability is not the cause of the decanalization, which instead happens as an indirect side effect of changes in allele frequencies due to shifts in the trait mean. Except in cases in which environmental fluctuations create systematic convexities in the local fitness landscape, there will be similar canalizing selection as generated by pure stabilizing selection. The main difference is that adaptive canalization is more easily disturbed in fluctuating environments. Hence, if evolvability is to evolve as an adaptation to fluctuating environments this must happen by other mechanisms such as group selection or selection for favorable trait correlations.

Acknowledgments This work was supported by the Norwegian Research Council through grant 177857 to TFH, the European Research Council through grant ERG 256507 to ALR, and the Spanish Ministry of Science and Innovation through grant BFU2010-20003 to JMAC.

Appendix 1: Multiplicative Selection

Here we derive some base-line predictions for the long-term multiplicative fitness function under different types of fluctuating selection. Consider first Gaussian selection around a fluctuating optimum

$$W(z;t) = k_t \,\mathrm{Exp}\Big[-s(z-\theta_t)^2\Big], \tag{14}$$

where θ_t is the value of the optimum in generation t and k_t an arbitrary time-dependent variable. The multiplicative relative fitness over T generations is then

$$\begin{aligned}
&\mathrm{Exp}\Bigg[\sum_t Ln[k_t] - \sum_t Ln[\overline{W}(t)] - sT(z-\overline{\theta})^2 \\
&\quad -2s(z-\overline{\theta})\sum_t(\theta_t-\overline{\theta}) - s\sum_t(\theta_t-\overline{\theta})^2\Bigg] \\
&= Exp\Bigg[\sum_t Ln[k_t] - Ln\big[\overline{W}(t)\big]\Bigg] Exp\Big[-sT(z-\overline{\theta})^2 \\
&\quad +2(z-\overline{\theta})\sum_t(\theta_t-\overline{\theta})/T + \sum_t(\theta_t-\overline{\theta})^2/T\Bigg],
\end{aligned} \tag{15}$$

where $\bar{\theta}$ is the mean optimum over the fluctuations. In the limit when $T \to \infty$ this reduces to

$$\begin{aligned}
\underset{T\to\infty}{Lim}\prod_t \frac{W(z;t)}{\bar{W}(t)} &= KExp\Big[-sT(z-\bar{\theta})^2 + Var[\theta]\Big] \\
&\sim Exp\Big[-sT(z-\bar{\theta})^2\Big],
\end{aligned} \tag{16}$$

where K is a constant and Var[θ] is the variance of the optimum over the fluctuations. Hence, the multiplicative relative fitnesses depend only on the average of the fluctuating optimum and not on the details of its distribution. They are the same with constant and varying optima, and we predict that the long-term effects of fluctuating gaussian optima should be similar to long-term stabilizing selection. If also the strength of selection is fluctuating, the situation is slightly more complicated. In this case

$$\underset{T\to\infty}{Lim}\prod_t \frac{W(z;t)}{\bar{W}(t)} \sim Exp\Big[-\bar{s}T\Big((z-\bar{\theta})^2 + 2T(z-\bar{\theta})Cov[s_t,\theta_t]\Big)\Big], \tag{17}$$

where $\bar{s} = \Sigma_t s_t/T$ is the average strength of selection. Hence, if there is a covariance between the changes of the optimum and the strength of stabilizing selection on that optimum, then this will induce a component of selection in the direction in which the deviances are associated with stronger selection. This shifts the long-term optimum to $\bar{\theta} + \mathrm{Cov}[s_t, \theta_t]/\bar{s}$. Note that this implies that there are no effects of fluctuations in the strength of selection if the optimum is fixed.

In this argument we can see that the extra stabilizing selection induced by variance in the selection gradient (Eq. 3) is compensated by the fluctuations shifting the population into more convex areas of the fitness landscape, which happens because the Gaussian fitness function is most concave at the optimum. If we consider quadratic stabilizing selection of the form

$$W(z;t) = k_t\Big(1 - s(z-\theta_t)^2\Big), \tag{18}$$

the first and second derivatives are $-2s(z-\theta_t)$ and $-2s$. Hence, from equation 1 we see that the second derivative of the multiplicative fitness function will be proportional to $-2s - 4s^2\mathrm{Var}[\theta_t]$, which implies that the strength of stabilizing selection will increase with the variance of fluctuations in the optimum (as long as the occurence of non-positive fitnesses is negligible).

Similar effects happen with fluctuating directional selection. Consider first a fitness function of the form

$$W(z;t) = k_t(1 + s_t z), \tag{19}$$

where s_t fluctuates such that the population experience no net long-term directional selection. In this case the long-term multiplicative function has a second derivative proportional to $-\Sigma_t s_t^2/(1+s_t z)^2$, which implies that the strength of stabilizing selection will increase with increasing fluctuations in s_t. The optimum will depend on the distribution of s_t, but if this has mean zero, the optimum will be at $z = 0$. With exponential directional selection ($W(z;\, t) = \mathrm{Exp}[s_t z]$), which is inherently convex, the multiplicative fitness function will be flat if the s_t average to zero.

A slightly different result follows from an alternating linear fitness function of the type

$$W(z;t) = 1 + s_t(z - z_t), \tag{20}$$

where $\bar{z}_t$ is the mean of z in generation t. This form ensures that the selection gradient is always equal to s_t and is hence a model of a fluctuating linear selection gradient. In this case the mean fitness in each generation is always unity, so that the cumulative fitness function is

$$\begin{aligned}
\prod_t \frac{W(z;t)}{\bar{W}(t)} &= \prod_t (1 + s_t(z_t - \bar{z}_t)) \\
&= Exp[\Sigma_t Ln[1 + s_t(z_t - \bar{z}_t)]]
\end{aligned} \tag{21}$$

By assuming no net directional selection in the sense that $\bar{s} = 0$, that selection within each generation is weak in the

sense that $|s_t(z-\bar{z}_t)|<1$, and considering only the two first terms in a series expansion of the logarithm we get

$$\prod_t \frac{W(z;t)}{\bar{W}(t)} \sim Exp\left[-\frac{1}{2}T\sigma_s^2(z-\bar{z})\right], \tag{22}$$

where σ_s^2 is the temporal variance of the selection coefficient and $\bar{z}=\Sigma_t\bar{z}_t/T$ is the mean phenotype over the history of the population. Hence, the multiplicative fitness landscape approximates normalizing selection around the average mean phenotype during the period we consider. This resembles "normalizing" selection (Travis 1989) favoring the current mean. Even if there is no net directional selection, slight random changes in the mean will be preserved, so that the mean can slowly drift away from its ancestral value in a Brownian-motion-like manner. To understand how this normalizing selection arises, consider a situation in which the selection alternates every generation between positive and negative, but is always equally strong such that the mean shifts back and forth between two values m_1 and m_2. In this situation phenotypic values intermediate between m_1 and m_2 will always have higher fitness than the current mean.

Appendix 2: Selection on a Decanalizing Gene Substitution

In this appendix we compute the fitness effects of a canalizing or decanalizing substitution at a locus, x. The phenotype is

$$\begin{aligned} z &= z_r + \Sigma_i {}^i y + \Sigma_i\Sigma_{j>i} {}^{ij}\varepsilon {}^i y {}^j y + \Sigma_i\Sigma_{j>i}\Sigma_{k>j} {}^{ijk}\varepsilon {}^i y {}^j y {}^k y \\ &\quad + \cdots + {}^{g\to x}f\, x \\ &= z_{x=0} + {}^{g\to x}f\, x \end{aligned} \tag{23}$$

where x is the reference effect of the substitution, $z_{x=0}$ is the phenotype when $x=0$, and the epistasis factor, ${}^{g\to x}f = 1 + \Sigma_i {}^{ix}\varepsilon {}^i y + \Sigma_i \Sigma_{j>i} {}^{ijx}\varepsilon {}^i y {}^j y + \cdots$, describes the epistatic effects on x from the rest of the genome, $g=\{{}^1y,\ldots,{}^ny\}$. Hence, the term ${}^{g\to x}fx$ describes the effect of the substitution in the genetic background of g. We will study a substitution with effect $x=1$. This sets a scale and entails no loss of generality. We allow epistasis of any order. Assuming a quadratic fitness function $W(z)=1-s(z-\theta)^2$, the change from $x=0$ to $x=1$ gives a fitness change of

$$\begin{aligned} &\mathrm{E}_g[W(z)|x=1]-\mathrm{E}_g[W(z)|x=0] \\ &\quad = -s\mathrm{E}_g\left[2^{g\to x}f(z_{x=0}-\theta)+{}^{g\to x}f^2\right], \end{aligned} \tag{24}$$

where E_g refers to expectations taken over the reference effects of the loci in g. Assuming linkage equilibrium, this can be written

$$\begin{aligned} &\mathrm{E}_g[W(z)|x=1]-\mathrm{E}_g[W(z)|x=0] \\ &\quad = -s\mathrm{E}_g\left[2^{g\to x}f(z-\theta)+{}^{g\to x}f^2(1-2\bar{x})\right] \\ &\quad = -2s(\bar{z}-\theta)\mathrm{E}_g[{}^{g\to x}f] - 2s\mathrm{Cov}_g[z,{}^{g\to x}f] \\ &\quad - s(1-2\bar{x})\left(\mathrm{E}_g[{}^{g\to x}f]^2+\mathrm{Var}_g[{}^{g\to x}f]\right) \\ &\quad = -2s(\bar{z}-\theta)\mathrm{E}_g[{}^{g\to x}f] - 2s\mathrm{Cov}_g[z_{x=0},{}^{g\to x}f] \\ &\quad - s\mathrm{Var}_g[{}^{g\to x}f] - s(1-2\bar{x})\mathrm{E}_g[{}^{g\to x}f]^2, \end{aligned} \tag{25}$$

where the last step uses $\mathrm{Cov}_g[z,\ {}^{g\to x}f]=\mathrm{Cov}_g[z_{x=0}+{}^{g\to x}f\,x,\ {}^{g\to x}f]=\mathrm{Cov}_g[z_{x=0},\ {}^{g\to x}f]+\bar{x}\mathrm{Var}_g[{}^{g\to x}f]$.

In the bilinear case we can write

$$\mathrm{Cov}_g[z_{x=0},{}^{g\to x}f]=\sum_i {}^{ix}\varepsilon\mathrm{E}_g[{}^{g\to x}f]{}^iv, \tag{26a}$$

$$\mathrm{Var}_g[{}^{g\to x}f]=\Sigma_i {}^{ix}\varepsilon^2\,{}^iv, \tag{26b}$$

where ${}^iv=\mathrm{Var}[{}^iy]$, and ${}^{g\to x}f=1+\Sigma_j {}^{ij}\varepsilon\,{}^jy$. This yields Eq. 7 in the main text. Returning to the general multilinear case, we simplify the equations by using $\bar{g}=\{{}^1\bar{y},\ldots,{}^n\bar{y}\}$ and $x=0$ as reference genotype. Then

$$\begin{aligned} \mathrm{Cov}_g[z_{x=0},{}^{g\to x}f] &= \Sigma_i {}^{ix}\varepsilon^i v + \Sigma_i\Sigma_{j>i} {}^{ijx}\varepsilon^{ij}\varepsilon^i v^j v \\ &\quad + \Sigma_i\Sigma_{j>i}\Sigma_{k>j} {}^{ijkx}\varepsilon^{ijk}\varepsilon^i v^j v^k v + \cdots, \end{aligned} \tag{27a}$$

$$\begin{aligned} \mathrm{Var}_g[{}^{g\to x}f] &= \Sigma_i {}^{ix}\varepsilon^2\,{}^iv + \Sigma_i\Sigma_j {}^{ijk}\varepsilon^2\,{}^iv^j v \\ &\quad + \Sigma_i\Sigma_j\Sigma_k {}^{ijkx}\varepsilon^2\,{}^iv^j v^k v + \cdots, \end{aligned} \tag{27b}$$

and then

$$\begin{aligned} &\mathrm{E}_g[W(z)|x=1]-\mathrm{E}_g[W(z)|x=0] \\ &\quad = -2s(\bar{z}-\theta) - 2s\mathrm{Cov}_g[z_{x=0},{}^{g\to x}f] \\ &\qquad - s\mathrm{Var}_g[{}^{g\to x}f] - s(1-2\bar{x}) \\ &\quad = -2s(\bar{z}-\theta) - s\Sigma_i {}^{ix}\varepsilon\left({}^{ix}\varepsilon+2\right){}^iv \\ &\qquad - s\Sigma_i\Sigma_j {}^{ijx}\varepsilon\left({}^{ijx}\varepsilon+{}^{ij}\varepsilon\right){}^iv^j v \\ &\qquad - s\Sigma_i\Sigma_j\Sigma_k {}^{ijkx}\varepsilon\left({}^{ijkx}\varepsilon+{}^{ijk}\varepsilon/2\right){}^iv^j v^k v \\ &\qquad - s(\text{Higher -order terms}) - s(1-2\bar{x}), \end{aligned} \tag{28}$$

where the higher-order terms have the form $\Sigma_J {}^{Jx}\varepsilon({}^{Jx}\varepsilon+{}^J\varepsilon/2^{k-2})\,\Pi_{j\in J}{}^jv$, where J is the set of all k-tuples of indices from g. Restricting to pairwise epistasis yields Eq. 8 in the main text.

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