Epistasis in Polygenic Traits and the Evolution of Genetic Architecture under Stabilizing Selection

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abstract: We consider the effects of epistasis in a polygenic trait in the balance of mutation and stabilizing selection. The main issues are the genetic variation maintained in equilibrium and the evolution of the mutational effect distribution. The model assumes symmetric mutation and a continuum of alleles at all loci. Epistasis is modeled proportional to pairwise products of the single-locus effects. A general analytical formalism is developed. Assuming linkage equilibrium, we derive results for the equilibrium mutation load and the genetic and mutational variance in the house of cards and the Gaussian approximation. The additive genetic variation maintained in mutation-selection balance is reduced by any pattern of the epistatic interactions. The mutational variance, in contrast, is often increased. Large differences in mutational effects among loci emerge, and a negative correlation among (standard mean) locus mutation effects and mutation rates is predicted. Contrary to the common view since Waddington, we find that stabilizing selection in general does not lead to canalization of the trait. We propose that canalization as a target of selection instead occurs at the genic level. Here, primarily genes with a high mutation rate are buffered, often at the cost of decanalization of other genes. An intuitive interpretation of this view is given in the discussion.

Keywords: epistasis, mutation-selection balance, genetic architecture, canalization, mutational robustness, genetic variance, quantitative genetics.

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Most life-history, morphological, or behavioral characters are so-called complex quantitative traits; that is, they vary along a quantitative scale and are influenced by more than one or two genes (they are polygenic). A major challenge of current quantitative genetics is to elucidate the genetic architecture of these traits (for a recent review, see Mackay 2001) and to understand the evolutionary forces that shaped this architecture. Although epistasis appears to be a nearly universal component of the architecture of most quantitative traits (Templeton 2000) and has important evolutionary consequences (Fenster et al. 1997), it is often disregarded in population genetic studies (but see Wolf et al. 2000). In this article, we discuss a model of a quantitative character with multilinear epistasis among the underlying genes and analyze the effects of epistasis in mutation-selection balance. Two primary questions motivate our work: How does epistasis affect the maintenance of genetic variation in equilibrium? and How does stabilizing selection shape the genetic architecture of an epistatic trait?

For a purely additive trait, a highly developed mathematical theory exists that has succeeded in providing accurate estimates for the genetic variance in mutation-stabilizing-selection balance (see Bürger 2000 for a detailed account). Depending on the mutational parameters, two approximations apply. If mutation rates are low and/or mutational effects are sufficiently large, the genetic variance is mainly due to a few mutations of large effects that segregate in the population at equilibrium. This situation is described by Turelli's (1984) house of cards (HC) approximation. The Gaussian approximation of Lande (1976*b*), on the other hand, assumes a normal distribution of the allelic effects and requires high mutation rates and/or low mutational effects. The results show that reasonably high levels of genetic variance can be explained this way, although the estimates, in particular for traits under strong selection in the HC regime, are still lower than typically measured values. The additive model has been extended in various directions in order to include drift, the effect of different mating systems, or inbreeding (cf. Bürger 2000). The effect of epistasis, however, has so far only been

studied in some special models of balancing selection (Gimelfarb 1989; Gavrilets and de Jong 1993) but not for a polygenic trait in mutation-stabilizing-selection balance.

The term "genetic architecture" refers to the number, identities, and variational properties of the genes (or, more generally, the variationally independent loci) that participate in the development of a character. The variational properties include the locus mutation rates as well as the distribution of single and multiple mutational effects, which are determined by the nature of the genetic interactions, that is, epistasis and dominance (Lynch and Walsh 1998, p. 321). In contrast to the population genetic quantities that depend on the allele frequencies, the variational properties are properties of genotypes in a given environment. In this article, we will concentrate on the evolution of the mutational-effect distribution. For each given genotype, this distribution describes how mutational variation maps to phenotypic variation. For purely additive gene effects, it is independent of the genotype in which it is measured. In the presence of dominance or epistasis, it depends on the genetic background and may evolve.

The most frequently investigated ideas about the evolution of mutational effects are the evolution of dominance (reviewed in Mayo and Bürger 1997) and canalization (reviewed in Gibson and Wagner 2000; Hermisson and Wagner 2003). Canalization is a concept that goes back to Waddington (1953) and Schmalhausen (1949), who hypothesized that stabilizing selection should lead to the evolution of genotypes that are less sensitive to recurrent deleterious mutations. There is some preliminary empirical evidence for the existence of robust genotypes (reviewed in Scharloo 1991), and there are some theoretical arguments supporting the basic idea of stabilizing selection causing canalization (Wagner 1996; Wagner et al. 1997; van Nimwegen et al. 1999; and many more), although the results are far from convincing (Hermisson and Wagner 2003).

In this article, we present a detailed mathematical analysis of a rather general model (a polygenic trait with a continuum of alleles and epistasis proportional to products of the single-locus effects in the balance of symmetric mutations and stabilizing selection). Assuming linkage equilibrium, we show that the additive genetic variance that is maintained in equilibrium is reduced by any pattern of epistasis in the model, under Gaussian as well as under house of cards conditions; we also show that canalization on the level of the trait is not a necessary outcome of stabilizing selection and epistasis. In many cases, the average effects of loci diverge, leading to genes with large effects (major loci) and others with small effects (minor loci), rather than a uniform decrease of effects. Buffering effects are found primarily for genes with high mutation rates.

The first two sections of this article introduce the multilinear model (Hansen and Wagner 2001*b*) and set up the formalism. Central results in this part are the equilibrium condition (23) and the discussion of the selection forces. In the third section, the formalism is applied to the two-locus case as a concrete example where everything can be calculated explicitly. Subsequently, the multilocus results are presented. Two sections are devoted to the evolution of the genetic architecture and the effects of epistasis on the genetic variances. The "Discussion" summarizes the most important results and expounds their consequences. Additional and technical material is collected in the appendices.

Model

We consider the evolution of a quantitative trait and the underlying genetic architecture in a randomly mating diploid population with equivalent sexes. Population size is assumed to be sufficiently large to ignore random genetic drift. The phenotypic value is $z = x + e$, where the environmental component e is given by a random variable with zero mean and variance V_E. The environmental effects are assumed independent of the genotypic value x of an individual, which is given by the following map:

$$x = x_r + \sum_i y_i + \sum_{i,j>i} \varepsilon_{ij} y_i y_j. \tag{1}$$

Here, the single-locus variables $y_i = a_i + a_i^*$ are determined additively by the maternal and paternal effects. We thus neglect, for simplicity, dominance in the model. In the following, it will be convenient to directly calculate with whole-locus (diploid) quantities. Due to equivalence of the sexes, and assuming Hardy-Weinberg proportions throughout, the whole-locus cumulants are just twice the corresponding haploid ones. In particular, we have $\bar{y}_i = 2\bar{a}_i$ and $V_i = 2\,\mathrm{Var}(a_i)$ for the locus means and variances.

The functional form of the trait values (eq. [1]) is a special case of the multilinear model of gene interactions (Hansen and Wagner 2001*b*) with pairwise interactions only. The multilinear model was derived from the assumption that the genetic background influences the allelic effects at any given locus by a common factor. It is motivated by the interest of an operational definition of all model parameters on the level of the phenotype itself. The central concept to achieve this is the introduction of a reference genotype relative to which all allelic effects are measured. In this view, the y_i are the reference effects of the single-locus substitutions. They can assume any real value and span the genotype space. If there are n loci, a genotype (respectively, the class of genotypes with the same set of reference effects) is described by a vector $\boldsymbol{y} \in \mathbb{R}^n$

with components y_i. The reference genotype, in particular, is at the origin of the space. It is represented by the zero vector and has the genotypic value x_r. Deviations of the effects of multilocus substitutions from the sum of the single-locus effects give rise to the epistasis terms. For most of our study, only pairwise interactions with arbitrary epistasis parameters $\varepsilon_{ij} \in \mathbb{R}$ are included. An extension to higher-order interactions is discussed in appendix D.

An important role in the following will be played by the partial derivatives of x with respect to the reference effects:

$$f_i = f_i(\mathbf{y}) := \frac{\partial}{\partial y_i} x \bigg|_{\mathbf{y}} = 1 + \sum_{j \neq i} \varepsilon_{ij} y_j. \tag{2}$$

Here, $f_i(\mathbf{y})$ measures the sensitivity of the phenotype with respect to changes in the ith locus for individuals with genotype $\mathbf{y}$ relative to the reference; if the reference effect of a substitution is δ_i, its phenotypic effect in the background $\mathbf{y}$ is $f_i(\mathbf{y})\delta_i$. The f_i thus are epistasis factors (Hansen and Wagner 2001*a*, 2001*b*), capturing the epistatic effect of changes in the genetic background on the locus reference effects. As the genotypes, the epistasis factors also are conveniently represented in vector form. Defining $\nabla_{\mathbf{a}} b$ to be the gradient of b with respect to a set of variables a_i (i.e., the vector with ith component $\partial b/\partial a_i$), we can write

$$\mathbf{f} = \nabla_{\mathbf{y}} x = \mathbf{1} + \mathbf{E}\mathbf{y}, \tag{3}$$

where $\mathbf{1} = (1, 1, 1, \ldots)^t$ is the vector with all values as 1, and $\mathbf{E}$ is the epistasis matrix (Rice 1998), with elements

$$E_{ij} = \varepsilon_{ij} = \frac{\partial^2 x}{\partial y_i \partial y_j}. \tag{4}$$

Note that the diagonal elements of $\mathbf{E}$ are all 0.

Mutations at the ith locus occur at a diploid (twice the haploid) rate u_i and add a random increment δ_i to the locus reference effect y_i. The distribution of mutational reference effects is assumed to be symmetric with variance γ_i^2 and mean 0. Note that, in an epistatic model, the reference effect of any given mutation must be distinguished from its population mean effect. Since phenotypic effects in a reference background define the genotypes in this model, the mutational reference effects really describe mutations on a genotypic level and are fixed. The population mean phenotypic effect of a mutation δ_i, on the other hand, depends on the distribution of genetic backgrounds in the population. It is given by $\bar{f}_i\delta_i$ and can evolve. Similarly, the variance of phenotypic effects of mutations at the ith locus becomes $\langle f_i^2 \rangle \gamma_i^2$ in the population average. Here and in the following, we denote population means by angled brackets, but we use the overbar as a shorthand for single letters; that is, $\langle x \rangle = \bar{x}$. In order to clearly distinguish mean from reference effects, we will use the terms "mutational effect" and "mutational variance" exclusively in the meaning of these quantities in the population average. If, in contrast, reference effects or variances are referred to, this will be stated explicitly.

We assume weak quadratic viability selection of strength s toward an optimum x_{opt}. Malthusian (logarithmic) fitness is given by $m(z) = m_{\text{opt}} - s(z - z_{\text{opt}})^2$, which leads to a genotypic fitness of

$$m(x) = m_{\text{opt}} - sV_{\text{E}} - s(x - z_{\text{opt}})^2. \tag{5}$$

On a direct multiplicative scale, this corresponds to a Gaussian fitness function. By an appropriate change of the reference (e.g., the choice of $y_1 = z_{\text{opt}} - x_r$ and $y_i = 0$, $i > 1$ as coordinates of the reference genotype), we can always choose the genotypic value x_r of the reference genotype to coincide with the optimum trait value. In the following, we will therefore assume $x_r = z_{\text{opt}} = 0$ without loss of generality. Note, however, that a change of the reference also changes the epistasis coefficients and the mutational effects of the model according to

$$\delta_i' = f_i\delta_i, \; \varepsilon_{ij}' = \frac{\varepsilon_{ij}}{f_i f_j}. \tag{6}$$

If the phenotypic effects of allele substitutions in the new reference are small (small f_i), this can lead to large values of the epistasis coefficients.

An intuitive way to represent epistatic models is the picture of the phenotype landscape (Rice 1998), which expresses the phenotype as a function of underlying genetic factors, $x = x(y_1, \ldots, y_n)$.[1] A landscape illustration of the multilinear model (for two loci) is shown in figure 1. We do not suggest that the phenotype landscape of real traits are of this multilinear type. In the vicinity of an equilibrium, however, it may serve as good local approximation by capturing the first two terms of a Taylor expansion. On global scales, the important point that distinguishes the epistatic landscape from a purely additive model is that the variational properties (i.e., the effects of single and multiple mutants on phenotype) are able to change as the population evolves on the landscape. The multilinear model captures this and offers the population at any location in the landscape a broad variety of neighborhoods with different variational properties for natural selection to choose from. In particular, if the population evolves

[1] For simplicity, we will frequently refer to the genotypic value as the phenotype.

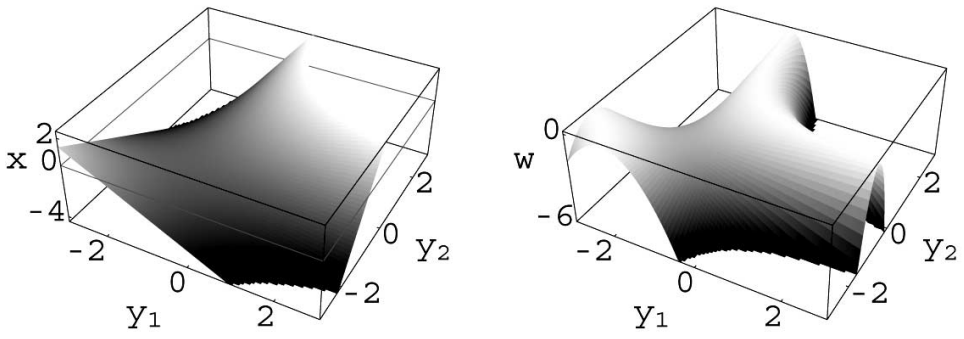

Figure 1: Phenotype landscape (*left*) and fitness landscape (*right*) of the multilinear model with two loci. On a phenotype landscape, genotypic values correspond to height and single-locus variational effects to slope. Epistatic effects manifest as curvature and are measured by the higher-order derivatives. On the fitness landscape, contours of equal fitness form a hyperbola-shaped ridge with the contour of the optimum phenotype, $x = 0$, at the top. The ridge is relatively flat in the vicinity of the reference genotype $y_1 = y_2 = 0$ but becomes steep as we follow the contour in either direction.

along subspaces of genotypes that lead to the same phenotype (called isophenotype contours in the following), it may change the variational properties without changing the genotypic value.

Expressions for Genetic Load and Variances

For the following, it will be convenient to express the phenotypic means and variances in terms of the mean epistasis factors $\bar{f}_i$ acting on the loci. In linkage equilibrium, the population mean can be written as $\bar{x} = \langle \mathbf{1}^t \boldsymbol{y} + \boldsymbol{y}^t \mathbf{E} \boldsymbol{y}/2 \rangle = \mathbf{1}^t \bar{\boldsymbol{y}} + \bar{\boldsymbol{y}}^t \mathbf{E} \bar{\boldsymbol{y}}/2$, where t denotes transposition. If $\mathbf{E}$ is invertible, we can use $\bar{\boldsymbol{y}} = \mathbf{E}^{-1}(\bar{\boldsymbol{f}} - \mathbf{1})$ to rewrite this relation as

$$\bar{x} = \frac{1}{2}\left(\bar{\boldsymbol{f}}^t \mathbf{E}^{-1} \bar{\boldsymbol{f}} - \mathbf{1}^t \mathbf{E}^{-1} \mathbf{1}\right). \tag{7}$$

The genetic variance V_G may be decomposed into its additive and epistatic part. Since we only consider pairwise interactions and we neglect dominance, the epistatic variance is entirely due to its additive × additive component. We thus have $V_G = V_A + V_{AA}$. In linkage equilibrium, V_A and V_{AA} may be expressed as

$$V_A = \bar{\boldsymbol{f}}^t \mathbf{V} \bar{\boldsymbol{f}} = \sum_i \bar{f}_i^2 V_i, \tag{8}$$

$$V_{AA} = \frac{1}{2} \mathrm{tr}[(\mathbf{EV})^2] = \frac{1}{2} \sum_i \mathrm{Var}\,(f_i) V_i = \sum_{i,j<i} \varepsilon_{ij}^2 V_i V_j. \tag{9}$$

Here, $\mathbf{V}$ is the variance matrix, which holds the locus variances V_i in the diagonal, and tr[$\mathbf{A}$] denotes the trace of $\mathbf{A}$. Along with other quantitative parameters of the multilinear model, these expressions were first derived in Hansen and Wagner (2001*b*, p. 68). Similar relations exist for higher moments of the phenotype distribution. For a symmetric distribution of all locus reference effects y_i (e.g., at the equilibria; see below), the third cumulant, for example, reads

$$C_3(x) = 3 \bar{\boldsymbol{f}}^t \mathbf{V} \mathbf{E} \mathbf{V} \bar{\boldsymbol{f}} + \mathrm{tr}[(\mathbf{EV})^3]. \tag{10}$$

Since fitness is quadratic in the trait values, the mutation load is simply

$$L = s(\bar{x}^2 + V_G) = s\left(\bar{x}^2 + \sum_i \bar{f}_i^2 V_i + \sum_{i,j<i} \varepsilon_{ij}^2 V_i V_j\right). \tag{11}$$

The mutational variance, finally, takes the form

$$\begin{aligned} V_m &= \sum_i V_{m,i} = \sum_i \sigma_{m,i}^2 \langle f_i^2 \rangle \\ &= \sum_i \sigma_{m,i}^2 \left(\bar{f}_i^{\,2} + \sum_{j \neq i} \varepsilon_{ij}^2 V_j\right), \end{aligned} \tag{12}$$

where $\sigma_{m,i}^2 = u_i \gamma_i^2$ is the mutational variance at the ith locus in the reference background.

Mutation-Selection Dynamics

Let us now consider the change in the distribution of genotypes under mutation and selection. The per-generation change of the means and variances of the single-locus reference effects, $\bar{y}_i$ and V_i, is twice the change of the corresponding haploid quantities, which is given by the Price equations (Price 1970; Frank and Slatkin 1990). In linkage equilibrium and for weak mutation and selection, the whole-locus changes read

$$\Delta\bar{y}_i = 2\Delta\bar{a}_i = 2\,\mathrm{Cov}(a_i, w),$$

$$\Delta V_i = 2\Delta\,\mathrm{Var}(a_i) = 2\,\mathrm{Cov}[(a_i - \bar{a}_i)^2, w] + \sigma^2_{\mathrm{m},i}. \quad (13)$$

From equation (13), the following relations for the change of the means and the variances of the locus reference effects may be derived (Turelli and Barton 1990):

$$\begin{aligned}\Delta\bar{y}_i &= V_i\frac{\partial}{\partial\bar{y}_i}\bar{w} + C_{3,i}\frac{\partial}{\partial V_i}\bar{w}\\ &= -2sV_i\left(\bar{x}\bar{f}_i + \sum_{j\neq i}\varepsilon_{ij}V_j\bar{f}_j\right) - sC_{3,i}\left(\bar{f}_i^{\,2} + \sum_{j\neq i}\varepsilon^2_{ij}V_j\right), \quad (14)\\ \Delta V_i &= (V_i^2 + C_{4,i})\frac{\partial}{\partial V_i}\bar{w} + C_{3,i}\frac{\partial}{\partial\bar{y}_i}\bar{w} + \sigma^2_{\mathrm{m},i}\\ &= -s(V_i^2 + C_{4,i})\left(\bar{f}_i^{\,2} + \sum_{j\neq i}\varepsilon^2_{ij}V_j\right) \quad (15)\\ &\quad - 2sC_{3,i}\left(\bar{x}\bar{f}_i + \sum_{j\neq i}\varepsilon_{ij}V_j\bar{f}_j\right) + \sigma^2_{\mathrm{m},i}.\end{aligned}$$

This set of equations cannot in general be solved because of its dependence on the third and fourth cumulant, $C_{3,i}$ and $C_{4,i}$, of the distribution of locus reference effects y_i. An important simplification occurs, however, if we concentrate on mutation-selection balance. Due to the multilinearity of the model, the marginal fitness at each locus is always quadratic around an optimum that depends on the mean genetic background experienced by this locus. Since the mutation distribution is also symmetric by assumption, all equilibrium distributions with finite densities at all loci are symmetric. (This follows, under reasonable assumptions on the mutation distribution, from the uniqueness of the solution of the haploid mutation-selection model [cf. Bürger 2000, p. 127]. This does not imply uniqueness of the solution in the multilocus case, however.) Due to epistasis, a symmetric distribution at all loci does not imply a symmetric distribution at the level of the phenotype (eq. [10]). Since all odd cumulants beyond the first one of a symmetric distribution vanish, we may set $C_{3,i} = 0$ in mutation-selection equilibrium.

In order to close the equations for the variances, we must find approximate expressions for the fourth-order cumulant. There are two standard ways to do this. In the Gaussian allelic approximation (Lande 1976*a*), the allelic distribution at each locus is assumed to be normal, such that $C_{4,i}$ vanishes. This results in the equilibrium condition

$$V_i^2\langle f_i^2\rangle = V_i^2\left(\bar{f}_i^{\,2} + \sum_{j\neq i}\varepsilon^2_{ij}V_j\right) = \frac{\sigma^2_{\mathrm{m},i}}{s}. \quad (16)$$

In the house of cards (HC) approximation (Turelli 1984; Barton and Turelli 1987; Bürger and Hofbauer 1994), on the other hand, the allelic distribution is assumed to be highly leptokurtic, and the fourth-order cumulant is approximated $C_{4,i} \approx V_i\gamma_i^2 \gg V_i^2$. The equilibrium condition under HC conditions assumes the following form:

$$V_i\langle f_i^2\rangle = V_i\left(\bar{f}_i^{\,2} + \sum_{j\neq i}\varepsilon^2_{ij}V_j\right) = \frac{u_i}{s}. \quad (17)$$

The Gaussian and HC approximations are valid in different regions of parameter space. The HC conditions apply if the phenotypic variance of the new mutations at a locus is (much) larger than the equilibrium genetic variance. In particular, it can be shown that the HC approximation is exact to leading order in u_i/s in the limit $u_i \to 0$ (Bürger 2000, chap. 6). The Gaussian allelic approximation, on the other hand, requires mutational effects to be small (or mutation rates to be high). In an epistatic model, the phenotypic effect of a given mutation depends on the genetic background. The variance of these effects, $\langle f_i^2\rangle\gamma_i^2$, is free to evolve. The appropriate approximation therefore depends on the position of the population on the phenotype landscape. From equations (16) and (17) we see that both approximations coincide if $\langle f_i^2\rangle\gamma_i^2 = u_i/s$. Numerical studies for a normal mutation distribution show that the HC approximation is better than the Gaussian allelic approximation if $\langle f_i^2\rangle\gamma_i^2 > u_i/s$, and vice versa (Bürger 2000, pp. 239 ff.). We will therefore apply either approximation, depending on the evolvable value of $\langle f_i^2\rangle\gamma_i^2$, and refer to the respective regions on the phenotype landscape as the Gaussian and the house of cards mutation-selection regime.

Interpretation of Dynamical Equations

In equations (14) and (15), the changes of the locus means and variances due to selection on the trait are expressed in terms of selection gradients (Barton and Turelli 1987) proportional to derivatives of the mean fitness. Following Rice (1998, 2000), we may now analyze the selective forces that drive this evolutionary dynamics. To this end, we expand the mean fitness as $\bar{w} = -s(\bar{x}^2 + V_{\mathrm{G}})$ and consider the resulting terms in equations (14) and (15) separately. This results in three forces, given by the gradients

$$\mathcal{F}^{\mathrm{a}} := -s\nabla_{\bar{y}}\bar{x}^2 = \frac{\partial}{\partial\bar{x}}w(\bar{x})\bar{\boldsymbol{f}} = -2s\bar{x}\bar{\boldsymbol{f}}, \quad (18)$$

$$\mathcal{F}^{\varepsilon} := -s\nabla_{\bar{y}}V_{\mathrm{G}} = -s\nabla_{\bar{y}}V_{\mathrm{A}} = -2s\mathbf{E}\mathbf{V}\bar{\boldsymbol{f}}, \quad (19)$$

$$\mathcal{F}^{\mathrm{V}} := -s\nabla_{\mathrm{V}}V_{\mathrm{G}} = -s[\bar{f}_i^{\,2} + \mathrm{Var}(f_i)]. \quad (20)$$

Here, **E** is the epistasis matrix, defined in equation (4), and **V** is the covariance matrix of reference effects $V_{ii} = V_i$. The forces enter the mutation-selection response as

$$\Delta\bar{y}_i = V_i(\mathcal{F}_i^{\mathrm{a}} + \mathcal{F}_i^{\varepsilon}) + C_{3,i}\mathcal{F}_i^{\mathrm{V}}, \tag{21}$$

$$\Delta V_i = (V_i^2 + C_{4,i})\mathcal{F}_i^{\mathrm{V}} + C_{3,i}(\mathcal{F}_i^{\mathrm{a}} + \mathcal{F}_i^{\varepsilon}) + \sigma^2_{\mathrm{m},i}. \tag{22}$$

The symbol $\mathcal{F}^{\mathrm{a}}$, which we will call the adaptive force, describes the part of the dynamics that corresponds to the classical result for an additive trait; that is, selection acts as a force proportional to the change in fitness. The locus means evolve at a rate proportional to the locus genetic variance and the slope of the fitness function in that direction.

The directional epistatic force $\mathcal{F}^{\varepsilon}$, on the other hand, vanishes in the additive model where changes in the locus means have no effect on the genetic variance. It is this new force which primarily drives the evolution of the genetic architecture on an epistatic phenotype landscape. Force $\mathcal{F}^{\varepsilon}$ points in the direction of the gradient of the genetic variance (or the additive genetic variance). It thus drags the locus means into the direction of a reduced landscape slope in order to minimize the phenotypic effect of the genetic variation that is present in the population.

We have argued above that the distribution at all loci must be symmetric in mutation-selection balance. As may be seen from equation (21), the forces $\mathcal{F}^{\mathrm{a}}$ and $\mathcal{F}^{\varepsilon}$ are therefore the only ones that act on the locus means in the vicinity of equilibria, and the equilibrium condition is just that the two forces balance. We can express this as an eigenvalue equation of the operator **EV**,

$$\mathbf{EV}\bar{\boldsymbol{f}} = -\bar{x}\bar{\boldsymbol{f}}. \tag{23}$$

The simple structure of this equation will greatly promote our analysis in the following.

Finally, the only selective force on the variances that contributes in the vicinity of an equilibrium is $\mathcal{F}^{\mathrm{V}}$. In the absence of mutation, this force simply drives the variances to 0. With mutation, it is balanced by the new mutational input. In the different mutation-selection regimes (Gaussian, HC), different approximations apply for the factor $(V_i^2 + C_{4,i})$ that weights selection relative to mutation (see eqq. [16] and [17]).

Two Loci

Let us begin the analysis of the equilibrium properties with a detailed discussion of the two-locus case, $x = y_1 + y_2 + \varepsilon y_1 y_2$. For this simple model system, the equilibrium condition is readily evaluated, and explicit expressions for all population level quantities can be derived. The model is nevertheless also informative of the general case. We will see below that many characteristic properties extend to models with multiple loci.

As may be seen from figure 1, the phenotype and fitness landscapes of the model are point symmetric. The center of symmetry is the point $y_1 = y_2 = -1/\varepsilon$, or $f_1 = f_2 = 0$. Since the mutational part also is symmetric, equilibrium solutions will come in symmetry pairs (related by $\bar{f}_{1,2} \to -\bar{f}_{1,2}$), which allows us to restrict the analysis to the half-plane, $\bar{f}_1 \geq 0$.

Where does a population on this landscape evolve under the action of mutation and selection? From an analysis of the dynamical equations, two different types of equilibria are found, which we will now discuss in turn.

Balancing-Forces Equilibrium

As we have seen in the last section, two forces, the adaptive force $\mathcal{F}^{\mathrm{a}}$ and the epistatic force $\mathcal{F}^{\varepsilon}$ act on the locus means in the vicinity of an equilibrium. Suppose now that epistasis is weak. In this case, $\mathcal{F}^{\mathrm{a}}$ is much stronger than $\mathcal{F}^{\varepsilon}$ almost everywhere on the phenotype landscape, and the population mean is driven toward the optimal trait value. We therefore expect to find the equilibria located near the contour of the optimal phenotype. However, since the epistatic force does not vanish at $\bar{x} = 0$, the equilibrium mean phenotype will slightly deviate from the optimum. Since $\mathcal{F}^{\varepsilon}$ seeks to reduce the genetic variance, it pulls the population toward the side where the phenotype landscape is flatter (i.e., $\bar{x} < 0$ if $\varepsilon > 0$) until the two forces $\mathcal{F}^{\mathrm{a}}$ and $\mathcal{F}^{\varepsilon}$ balance. According to equation (23), this is just the case if $-\bar{x}$ is an eigenvalue of **EV**. In order to determine the exact position of equilibria on the slope of the ridge, the two different approximations for the variances (Gaussian and HC) must be treated separately. Calculations are given in appendix A.

Gaussian. For given mutational variances $\sigma^2_{\mathrm{m},1}$ and $\sigma^2_{\mathrm{m},2}$ in the reference genotype, the following equilibrium values for trait means and variances are derived in the Gaussian approximation:

$$\begin{aligned}
\bar{x} &= -\varepsilon\sqrt{\sigma_{\mathrm{m},1}\sigma_{\mathrm{m},2}/s},\\
L &= 2\sqrt{s\sigma_{\mathrm{m},1}\sigma_{\mathrm{m},2}},\\
V_{\mathrm{A}} &= 2\sqrt{\frac{\sigma_{\mathrm{m},1}\sigma_{\mathrm{m},2}}{s}} - 2\varepsilon^2\frac{\sigma_{\mathrm{m},1}\sigma_{\mathrm{m},2}}{s},\\
V_{\mathrm{AA}} &= \varepsilon^2\frac{\sigma_{\mathrm{m},1}\sigma_{\mathrm{m},2}}{s},\\
V_{\mathrm{m}} &= 2\sigma_{\mathrm{m},1}\sigma_{\mathrm{m},2}.
\end{aligned} \tag{24}$$

The equilibrium exists whenever V_A is nonnegative, which restricts the epistasis coefficient to $\varepsilon^2 \leq (s/\sigma_{m,1}\sigma_{m,2})^{1/2}$. The use of the Gaussian approximation at the equilibrium is consistent (i.e., $\langle f_i^2\rangle\gamma_i^2 < u_i/s$; see above) if $(\sigma_{m,1}\sigma_{m,2}/s)^{1/2} < u_{1,2}/s$.

We see that the deviation of the trait mean from the contour of the optimal phenotype becomes very small for small ε. On the contour defined by $\bar{x}$, the equilibrium is located at the point where the mutational variances at both loci take the same value, $V_{m,1} = V_{m,2} = \sigma_{m,1}\sigma_{m,2}$. If the locus mutation rates differ, the locus with the smaller rate compensates for this by evolving the larger variance of mutational effects, $\langle f_i^2\rangle\gamma_i^2$. The position of the equilibrium relative to the origin (on the scale of the y_i) depends on the strength of epistasis: for weak epistasis, equal values of the locus mutational variances are only reached at a larger distance from the origin.

The equilibrium mutational variance and the mutation load are independent of the epistasis coefficient. Nevertheless, both these quantities are reduced relative to a model without epistasis if $\sigma_{m,1} \neq \sigma_{m,2}$ (geometric vs. arithmetic means). In general, we thus do not recover an equilibrium solution of the additive model in the limit $\varepsilon \to 0$. The mathematical cause for this discontinuous behavior is the translation invariance of the additive model with random walk mutation. This leads to a high degeneracy of the equilibrium solution, with solutions on an $(n-1)$-dimensional manifold. As noted by Kimura (1981), this provides the possibility for extensive neutral evolution at individual loci in this model. The same property, however, makes the additive model structurally unstable with respect to the introduction of gene interactions. The old equilibria vanish and new equilibria emerge from infinity for arbitrarily small epistasis.

House of cards. A very different pattern of equilibrium solutions results if both loci are under HC conditions. Let us first consider equal locus mutation rates, $u_1 \equiv u_2$. In this case, we obtain a one-dimensional contour of degenerate equilibrium solutions, defined by $\bar{x} = -\varepsilon u_1/s$. For the load and the genetic variances, we derive

$$\begin{aligned} L &= 2u_1, \\ V_A &= 2u_1/s - 2\varepsilon^2 u_1^2/s^2, \\ V_{AA} &= \varepsilon^2 u_1^2/s^2. \end{aligned} \tag{25}$$

As in the Gaussian case, the solution exists whenever V_A is positive, that is, for $s \geq u_1\varepsilon^2$. Whereas the equilibrium values of the load and the genetic variances are invariant over the contour of degenerate equilibrium solutions, the mutational variance is highly variable. We find

$$V_m = \langle f_1^2\rangle\sigma_{m,1}^2 + \sigma_{m,2}^2/\langle f_1^2\rangle. \tag{26}$$

The mean squared epistasis factor $\langle f_1^2\rangle = \langle f_2^2\rangle^{-1}$ parameterizes the equilibrium contour. For consistency of the HC assumption, it must fall into the interval $\gamma_2^2/(u_1/s) > \langle f_1^2\rangle > (u_1/s)/\gamma_1^2$.

Assume now that the mutation rate at the second locus is higher, $u_2 > u_1$. If mutation rates at both loci are unequal, an equilibrium solution under HC conditions no longer exists (app. A). Instead, a population on the flatter slope of the fitness ridge evolves ever-increasing differences in the variances of locus mutational effects as $\bar{f}_1$ increases and $\bar{f}_2$ decreases. This may best be seen from the relation

$$\bar{f}_2\Delta\bar{f}_1 - \bar{f}_1\Delta\bar{f}_2 = 2s\varepsilon\bar{x}\left(V_1\bar{f}_1^2 - V_2\bar{f}_2^2\right), \tag{27}$$

which follows directly from equation (14) under the assumption of no skewness in the distribution of reference effects. Since $\varepsilon\bar{x}$ is negative and the equilibrium condition for the variances under HC conditions reads $V_1\bar{f}_1^2 - V_2\bar{f}_2^2 = (u_1 - u_2)/s$, the right-hand side of equation (27) is strictly positive if V_1 and V_2 are close to mutation-selection balance. While no equilibrium solution exists for unequal locus mutation rates as long as both loci are in the HC regime, this changes if we take into account that the mutation-selection regime may change itself as the genetic architecture evolves. Below, we consider the case of a mixed-regimes equilibrium with one locus under HC conditions and the other in the Gaussian regime.

Mixed regimes. If only the first locus is under HC conditions and not necessarily the second, we still obtain $\bar{x} = -\varepsilon u_1/s$ for any possible equilibrium. Also, the load and the genetic variances are constrained to the values given in equations (25). If we now assume that the second locus is under Gaussian conditions, we obtain an equilibrium solution if $\langle f_1^2\rangle = s\gamma_2^2 u_2/u_1$. This results in a mutational variance of

$$V_m = s\left(\gamma_1^2\gamma_2^2\frac{u_2}{u_1} + \frac{u_1^2}{s^2}\right). \tag{28}$$

The first locus is consistently in the HC regime if $\gamma_1^2\gamma_2^2(u_2/s) > (u_1/s)^3$. The Gaussian condition for the second locus, $\langle f_1^2\rangle^{-1}\gamma_2^2 < u_2/s$, translates into the condition $u_2 > u_1$ for the locus mutation rates. As in the Gaussian case, the equilibrium genetic variance and the mutation load in the epistatic model are reduced relative to an additive model. The total mutational variance, on the other hand, will usually be much higher at a mixed-regimes equilibrium. This occurs because the variance of mutational effects at the locus with the lower mutation rate becomes very large.

For a population that is initially in the HC regime with both loci, and $u_2 > u_1$, we obtain the following picture: after an initial movement along the ridge described above, the variance of mutational effects at the second locus becomes small enough for the Gaussian approximation to apply (at the point where both approximations produce equal values). Since in the Gaussian approximation, the expressed genetic variance at the locus, $V_2\langle f_2^2\rangle$, depends on the variance of mutational effects (other than in the HC regime), it will start to lag behind the HC value if $\langle f_2^2\rangle\gamma_2^2$ is reduced further. Eventually, this difference becomes large enough for the right-hand side of equation (27) to become 0, and the equilibrium is reached.

$V_A = 0$ *Equilibrium*

Suppose that epistasis is very strong. In this case, the directional epistatic force $\mathcal{F}^e$ will dominate the adaptive force $\mathcal{F}^a$ in large parts of the genotype space. Ignoring $\mathcal{F}^a$ for a moment, we see that $\mathcal{F}^e$ drags the locus means to the point $\bar{y}_1 = \bar{y}_2 = -1/\varepsilon$, where the landscape slope in the direction of both loci vanishes, $\bar{f}_1 = \bar{f}_2 = 0$. Since mutations no longer have a marginal effect at this point, the additive part of the genetic variance vanishes, $V_A = 0$. From equation (18), we also see that the adaptive force is 0 at this point, which therefore fulfills the equilibrium condition (23) of the means. Technically, $\bar{f}_1 = \bar{f}_2 = 0$ is the only solution to equation (23) where $-\bar{x}$ is not an eigenvalue of the operator **EV**. The latter has been the characteristic of the balancing-forces equilibrium above. Although the $V_A = 0$ equilibrium exists for arbitrary parameter values of the model, it is only stable for sufficiently large epistasis, as further discussed below.

The distance of the mean phenotype from the optimum in the $V_A = 0$ equilibrium follows from equation (7) and is given by $\bar{x} = -1/\varepsilon$, which is large for weak epistasis. Equation (10) shows that, nevertheless, the skewness of the distribution of the phenotype values always vanishes, in contrast to the balancing-forces equilibrium. In order to obtain the variances, the two approximation regimes must again be considered separately.

Gaussian. Solving equation (16) for V_1 and V_2 at $\bar{f}_i = 0$ and using the relations (8)–(12), we obtain the following results for the mutation load and the trait variances:

$$\begin{aligned} L &= \frac{s}{\varepsilon^2} + \left(s\varepsilon^2\sigma_{m,1}^2\sigma_{m,2}^2\right)^{1/3}, \\ V_G &= \left(\varepsilon\sigma_{m,1}\sigma_{m,2}/s\right)^{2/3}, \\ V_m &= 2\left(\varepsilon^4\sigma_{m,1}^4\sigma_{m,2}^4/s\right)^{1/3}. \end{aligned} \tag{29}$$

The system is consistently under Gaussian conditions if $V_G < u_{1,2}/s$, which is usually fulfilled if the epistasis coefficient ε is not very large. For very large ε, one or both loci at the $V_A = 0$ equilibrium cross over to the house of cards regime.

House of cards and mixed. As in the balancing-forces case, an equilibrium with both loci under HC conditions exists only for $u_1 = u_2$. This, again, leads to a degenerate set of equilibrium solutions that may be parameterized by $\langle f_1^2\rangle = (\varepsilon^2 u_1/s)/\langle f_2^2\rangle$. For consistency of the HC assumption, $\langle f_1^2\rangle$ must fulfill $(u_1/s)/\gamma_1^2 < \langle f_1^2\rangle < \varepsilon^2\gamma_2^2$. The load and the variances on the level of the phenotype are

$$\begin{aligned} L &= \frac{s}{\varepsilon^2} + u_1, \\ V_G &= \frac{u_1}{s}, \\ V_m &= \langle f_1^2\rangle\sigma_{m,1}^2 + \frac{\varepsilon^2 u_1/s}{\langle f_1^2\rangle}\sigma_{m,2}^2. \end{aligned} \tag{30}$$

For unequal mutation rates $u_2 > u_1$, we again obtain a mixed-regimes equilibrium with the second locus in the Gaussian regime. Expressions for means and variances are as above with the parameter $\langle f_1^2\rangle = \varepsilon^2\gamma_2^2 u_2/u_1$. The first locus remains under HC conditions as long as $u_1/s < (\varepsilon\sigma_{m,1}\sigma_{m,2}/s)^{2/3}$.

Equilibrium Structure

In the analysis above, we have described two types of equilibria in mutation-selection balance for weak and strong epistasis, respectively. In fact, the balancing-forces equilibria exist only for sufficiently weak epistasis (see above). An approximate stability analysis in appendix B indicates that they are locally stable whenever they exist. The same analysis shows that the $V_A = 0$ equilibrium is only stable if a balancing-forces equilibrium does not exist. Since these two types of equilibria exhaust all possible solutions of the equilibrium conditions for the means and the variances, no other equilibria can exist (assuming linkage equilibrium and either the HC or the Gaussian approximation). This indicates that there is exactly one stable equilibrium for any choice of the model parameters.

Numerical results for a normal mutation distribution show that both the HC and the Gaussian approximations overestimate the true locus genetic variance (cf. Bürger 2000, p. 240). The deviation is largest for $\langle\gamma_i^2\rangle \approx u_i/s$. We do not expect that the corrections lead to qualitative changes in the equilibrium structure, but they will lead to shifts in the positions of the equilibria. These shifts should be largest for the HC and mixed equilibria. For identical loci (equal mutation rates and reference variances), we

expect that higher-order correction terms to the HC approximation introduce very weak selection toward the symmetric point $\langle f_1^2 \rangle = 1$. This turns the degenerate contour of equilibrium solutions into an "almost degenerate" one. For $u_2 > u_1$, the "true" genetic variance at the second locus will be well below the HC value even before the variance of mutational effects is so small that the Gaussian approximation applies. The equilibrium therefore will be reached for somewhat smaller values of the parameter $\langle f_1^2 \rangle$ (i.e., closer to $\langle f_1^2 \rangle = 1$). The shift of the equilibrium point will be very small if $u_2 \gg u_1$, but it may be substantial if $u_2 < 2u_1$. Nevertheless, even small differences in the locus mutation rates will still lead to large differences in the equilibrium variances of mutational effects at both loci.

The equilibrium structure for the two-locus system with a general position of the optimal phenotype is summarized in figure 2. Note that the balancing-forces equilibrium always come in mirror pairs (according to $\bar{f}_i \to -\bar{f}_i$). The $V_A = 0$ equilibrium is only stable for very strong epistasis or weak selection or if the phenotype at the $V_A = 0$ equilibrium is very close to the optimal value, $z_{opt} \approx -1/\varepsilon$.

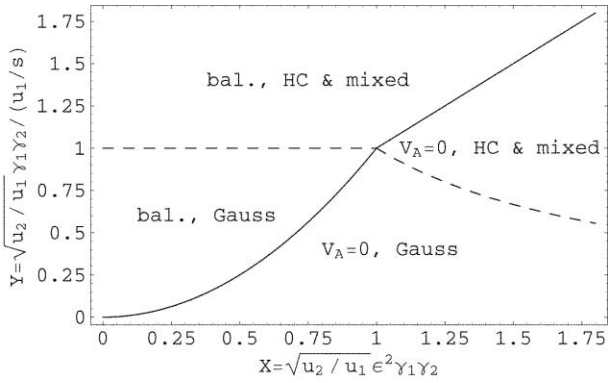

Figure 2: Stable equilibria of the two-locus model, with mutation rates $u_2 \geq u_1$ and optimal phenotype at $z_{opt} = 0$. The boundaries between the balancing-forces (*bal.*) and $V_A = 0$ equilibria are $Y = X^2$ (in the Gaussian regime [*Gauss*]) and $Y = X$ (in the house of cards [*HC*] or mixed regime). The $V_A = 0$ equilibrium is unstable whenever the balancing-forces equilibrium exists. The dashed lines $Y = 1$ and $Y = 1/X$ indicate the boundaries between the mutation-selection regimes. For a general optimal phenotype at $z_{opt} \neq 0$, the axes of the diagram must be rescaled like $X \to X/(1 + \varepsilon z_{opt})$ and $Y \to Y(1 + \varepsilon z_{opt})$, using the transformation rules (eqq. [6]).

Multiple Loci

Let us now consider how the two-locus results derived above generalize to a multilocus model. Explicit solutions for multilocus systems depend on the solvability of the eigenvalue equation (23), which quickly becomes intractable in higher dimensions for most epistasis patterns. Some general results are possible, however. Solutions for a number of special cases are given below and in appendix C.

General Properties of Equilibria

We have distinguished two types of equilibria in the analysis of the two-locus model, and it is easy to see that the same distinction also applies to the general case.

$V_A = 0$ *equilibrium.* An equilibrium with $\bar{f}_i \equiv 0, \forall i$ always exists if the epistasis matrix **E** is invertible. If **E** is singular, we show in appendix C that there is a $V_A = 0$ equilibrium if and only if the vector **1** is in the image of **E**. Otherwise, we obtain the mean phenotype at the $V_A = 0$ equilibrium from equation (7) as $\bar{x} = x_{V_A=0}$, where

$$x_{V_A=0} := \frac{1}{2}\sum_i \bar{y}_i = -\frac{1}{2}\mathbf{1}^t\mathbf{E}^{-1}\mathbf{1}, \tag{31}$$

which is independent of the mutation parameters u_i and γ_i^2. (If **E** is singular, but **1** is in the image of **E**, eq. [31] is evaluated in the vector space restricted to the image of **E**.) In the special case when all epistasis coefficients are equal, equation (31) gives $x_{V_A=0} = -n/[2(n-1)\varepsilon]$. In the HC approximation, we obtain the following simple expression for the genetic variance at a $V_A = 0$ equilibrium:

$$V_G = V_{AA} = \frac{1}{2}\sum_i \frac{u_i}{s}. \tag{32}$$

In the Gaussian and mixed regimes, this expression holds as an upper limit (cf. the balancing-forces case below).

Balancing-forces equilibria. For this type, the vector of mean epistasis factors $\bar{\boldsymbol{f}}$ is an eigenvector of **EV** and the corresponding eigenvalue is the negative mean phenotype $-\bar{x}$. A solution with the mean trait value at the optimum, $\bar{x} = 0$, is only possible if **E** is singular, that is, if the interaction patterns of a group of genes are linearly dependent. This means that there is redundancy among these genes, the simplest example being a pair of "duplicated" genes with identical epistasis patterns. If **E** is not singular, the directional epistatic force $\mathcal{F}^\varepsilon$ drags the mean phenotype away from its optimum into flatter regions of the landscape. In equilibrium, $\mathcal{F}^\varepsilon$ is balanced by the force $\mathcal{F}^a \propto -\bar{x}$, which tries to push the means back to the optimum. Using $V_A = -\bar{x}\bar{\boldsymbol{f}}^t\mathbf{E}^{-1}\bar{\boldsymbol{f}}$ in a balancing-forces equilibrium, we derive from equation (7)

$$\bar{x} = \frac{x_{V_A=0}}{2}\left(1 \pm \sqrt{1 - 2V_A/x_{V_A=0}^2}\right), \tag{33}$$

where $x_{V_A=0}$ is the trait mean in the $V_A = 0$ equilibrium (31). The sign $\pm$ in equation (33) depends on the values of the epistasis coefficients ε_{ij}. The sign of $\bar{x}$ always coincides with the sign of $x_{V_A=0}$, and $|\bar{x}| \leq |x_{V_A=0}|$. Whenever the model parameters are changed such that $\bar{x} \to x_{V_A=0}$, equation (33) shows that necessarily $V_A \to 0$. We thus obtain a continuous transition from a balancing-forces to the $V_A = 0$ equilibrium. The deviation from the contour of the optimal phenotype is small for weak epistasis (where the minus sign applies in eq. [33]). Under HC conditions, one expects a scaling behavior like $\bar{x} \sim \varepsilon u/s$ for small ε (assuming $u_i \propto u$ and $\varepsilon_{ij} \propto \varepsilon$). From equations (8) and (17), we see that the genetic variance in an epistatic system is always smaller than the one for an additive trait, since

$$V_G = \sum_i \frac{u_i}{s} - 2\sum_{i,j<i} \varepsilon_{ij}^2 V_i V_j = V_0 - V_{AA}, \tag{34}$$

where $V_0 = \sum_i u_i/s$ is the genetic variance in the absence of epistasis (remember that u_i is the diploid mutation rate). Similarly, $V_A = V_0 - 2V_{AA}$. In appendix D, we extend this result to higher-order interactions. We can combine equations (33) and (34) and obtain a simple relation for the equilibrium load

$$L = \frac{1}{2}\sum_i u_i + s\bar{x}x_{V_A=0}. \tag{35}$$

In the Gaussian regime, equation (34) holds as an upper limit since equations (8) and (16) give

$$V_G = \sum_i \frac{\gamma_i^2}{V_i}\frac{u_i}{s} - 2\sum_{i,j<i} \varepsilon_{ij}^2 V_i V_j \tag{36}$$

and $V_i \geq \gamma_i^2$ under Gaussian conditions. The same limit holds, of course, in mixed regimes. This bound does not necessarily imply a reduction of V_G relative to the additive trait under Gaussian conditions (see below for a counterexample).

Equilibrium structure. It is instructive to discuss how the constraints of the model affect its equilibrium structure. Since $\boldsymbol{f} = \mathbf{1} + \mathbf{E}\boldsymbol{y}$, and the variance of phenotypic effects of mutations at locus i is $\gamma_i^2 f_i^2$, any combination of these variances can evolve (if $\mathbf{E}$ is invertible). On a given isophenotype contour, however, only a subset of these combinations is possible. The central consequence of this constraint is that it keeps the $V_A = 0$ equilibrium and the optimal phenotype contour apart (except for a very special choice of the optimum). It thus introduces a trade-off between the evolutionary trend to optimize the phenotype (following the force $\mathcal{F}^a$) and the directional epistatic force $\mathcal{F}^\varepsilon = -s\nabla_{\bar{y}} V_A$ that drives the population toward the point $V_A = 0$ where the marginal effects of all mutations vanish.

Except for the case that s (and thus the fitness constraint) is very small, the $V_A = 0$ equilibrium will only be stable if epistasis is very strong. For decreasing epistasis, we show in appendix B that the $V_A = 0$ equilibrium becomes unstable precisely when a balancing-forces equilibrium (with smaller $|\bar{x}|$; cf. eq. [7]) appears. For given locus variances V_i, we further argue (but do not prove) that only the balancing-forces equilibrium with the smallest $|\bar{x}|$ can be stable. This suggests that the deviation of the mean phenotype from the optimum is usually small at stable equilibria. However, it does not imply that multiple stable equilibria cannot coexist since $\bar{x}$ also depends on the locus variances V_i. The modular model discussed below provides an example where stable equilibria with different $\bar{x}$ coexist.

Equivalent Loci

In this section, we derive explicit solutions for a special model with equivalent loci. This will allow us to take a closer look on the dependence of the equilibrium quantities on the number of loci, n, and on the relative strength of different components of epistasis. In order to characterize epistasis, we define a vector of directional epistasis with average epistasis coefficients ε_i as entries, as well as a coefficient for nondirectional epistasis Θ^2 as

$$\varepsilon_i := \frac{1}{V_A}\sum_{j\neq i} \varepsilon_{ij} V_j \bar{f}_j^{\,2},$$

$$\Theta^2 := \frac{1}{V_A^2}\sum_{i,j\neq i} \varepsilon_{ij}^2 V_i \bar{f}_i^{\,2} V_j \bar{f}_j^{\,2}. \tag{37}$$

These measures are averages of the bare epistasis coefficients, weighted by the contributions of individual loci to the additive genetic variance (cf. Hansen and Wagner 2001*b*). Let us now consider a model with equivalent loci, in the sense that $u_i \equiv u$, $\gamma_i \equiv \gamma$, and for all loci i:

$$\frac{1}{n}\sum_{j\neq i} \varepsilon_{ij} \equiv \varepsilon,$$

$$\frac{1}{n}\sum_{j\neq i} \varepsilon_{ij}^2 \equiv \theta^2. \tag{38}$$

The phenotypic means and variances can be derived for the symmetric equilibrium (i.e., $V_i \equiv V$ and $\bar{f}_i \equiv \bar{f}, \forall i$). Note that $\varepsilon_i \equiv \varepsilon$ and $\Theta^2 \equiv \theta^2$ at this point. Using $\bar{x} = -n\varepsilon V$, we obtain $\bar{f}^{\,2} = 1 - 2n\varepsilon^2 V$ from equation (7) and derive V from either equation (17) or equation (16). Under HC conditions, this results in

$$V = \frac{1}{2n(\Theta^2 - 2\varepsilon^2)}\left[\sqrt{1 + 4n(\Theta^2 - 2\varepsilon^2)u/s} - 1\right]$$
$$= \frac{u}{s} - n(\Theta^2 - 2\varepsilon^2)\frac{u^2}{s^2} + O[(u/s)^3], \quad (39)$$

where the last term is the error term with respect to epistasis as $u/s \to 0$. We obtain (with $U = nu$ the character mutation rate)

$$\bar{x} = -n\varepsilon V \quad (40)$$
$$= -\varepsilon\left\{\frac{U}{s} - (\Theta^2 - 2\varepsilon^2)\frac{U^2}{s^2} + O\left[(U/s)^3\right]\right\},$$

$$V_G = nV + \left(\frac{1}{2}\Theta^2 - 2\varepsilon^2\right)n^2V^2 \quad (41)$$
$$= \frac{U}{s} - \frac{1}{2}\Theta^2\frac{U^2}{s^2} + O\left[(U/s)^3\right],$$

$$V_A = nV - 2n^2\varepsilon^2V^2 = \frac{U}{s} - \Theta^2\frac{U^2}{s^2} + O\left[(U/s)^3\right], \quad (42)$$

$$V_m = [1 + (\Theta^2 - 2\varepsilon^2)nV]U\gamma^2 \quad (43)$$
$$= U\gamma^2\left\{1 + (\Theta^2 - 2\varepsilon^2)U/s + O\left[(U/s)^2\right]\right\}.$$

We see that the relative contribution of epistasis to all these quantities increases with the number of loci (i.e., it is proportional to U/s where U is the trait mutation rate). The main effect of directional epistasis is to drag the mean trait value away from the optimum. If there is strong directionality in epistasis (all epistasis coefficients ε_{ij} have the same sign), this shift may be quite large. In genetic architectures with a mix of positive and negative interactions, however, the contributions will in part compensate. This is different with the effect of nondirectional epistasis, which is mainly responsible for the effect of epistasis on V_G. Here, no compensation among positive and negative epistasis coefficients occurs. Comparing with the full solution of the two-locus case, we see that the low value of V_m in equation (43) is not representative but results from our restriction to the symmetric equilibrium.

Similar relations may also be derived under Gaussian conditions. Here, V is the solution of the cubic equation $n(\Theta^2 - 2\varepsilon^2)V^3 + V^2 - \sigma_m^2/s = 0$, and

$$V_G = v - v^2\varepsilon^2 + v^3\left(\frac{1}{8}\Theta^4 + \frac{1}{2}\Theta^2\varepsilon^2 + \frac{1}{2}\varepsilon^4\right) + O(v^4), \quad (44)$$

$$V_A = v - v^2\frac{1}{2}\left(\Theta^2 + 2\varepsilon^2\right) + O(v^3), \quad (45)$$

where $v = n\sigma_m/\sqrt{s}$ is the genetic variation in the absence of epistasis.

Some differences in the effect of directional and nondirectional epistasis relative to the HC case may be noted. We see that nondirectional epistasis may even lead to a slight increase of V_G while directional epistasis reduces this value. The additive genetic variance, on the other hand, is reduced by any kind of epistasis.

Modular Trait Structure

One plausible pattern of epistasis is a modular structure of gene interactions. It is natural to expect that interactions in groups of genes that regulate a shared pathway or depend upon each other in development are stronger than interactions between different subunits. Each subunit may describe, for example, the contribution of a particular gene product to the trait. We may also think of subunits as mutually pleiotropically independent characters underlying our focal trait. A modular trait structure may either be the result of evolution under natural selection or simply be fixed by developmental constraints.

Assuming modularity as given, we want to analyze its consequences for equilibria. To this end, we partition the loci in different groups. Effects of genes within a group or module may depend on each other through pairwise interactions, but there are no interactions (on the level of the trait) among groups. Note that this does not mean that the evolutionary dynamics of modules is independent, since of course there is epistasis for fitness between the groups.

Consider first a limiting case of such a modular structure where one "module" really consists only of a single interaction-free locus. Here, we immediately obtain a solution with $\bar{f}_1 = 1$, $\bar{f}_i = 0$, $i > 1$, if the first locus is interaction free and for arbitrary epistasis patterns among the other loci (such that the epistasis matrix restricted to the subspace of the other loci is invertible). These equilibrium values of the means appear to be stable independently of the values of the variances (cf. app. B). Obviously, what happens is the following: the interacting part of the system moves to the $V_A = 0$ equilibrium in its subspace, while the single free locus takes care of the trait optimization, compensating the phenotypic effects of all other loci. In equilibrium, $\bar{x} = 0$, $V_A = V_1$, and $L = s(V_1 + \sum_{i>1,\, j>i}\varepsilon_{ij}^2V_iV_j)$, with values of the variances depending on the mutation-selection regime. More generally, we obtain essentially this same behavior if the epistasis matrix has a zero eigenvalue in the subspace of any module. Since a single additive locus is sufficient to keep the trait mean at the optimum, this seems to be a generic situation. Note, however, that this result rests on the continuum-of-alleles assumption. If additivity is only maintained on an interval

of locus effects, or if the y_1 reach a limit that cannot be crossed without deleterious side effects, a single locus is no longer able to compensate the effects of all other loci.

For a general modular trait, we can dissect the eigenvalue equation for the means (eq. [23]) into equations on the subspaces corresponding to the modules. In all subspaces, we may separately decide to meet either the balancing-forces conditions (where $\bar{x}$ is an eigenvalue) or the $V_A = 0$ conditions (with all epistasis factors 0). The eigenvalues in all balancing-forces subspaces must coincide. For k modules, this procedure results in 2^k classes of equilibria that may exist and/or be stable depending on the model parameters. Note that only if the marginal effect for all loci in all subunits vanishes do we obtain a real $V_A = 0$ equilibrium as defined above. In appendix C, we explicitly solve the equilibrium structure for a system with two modules consisting of two loci each. We find that, at stable equilibria, one module takes care of the trait optimization and is at the balancing-forces equilibrium while the other evolves to $V_A = 0$ in its subspace.

Intuitively, we can interpret this behavior as follows. As explained above, an optimal trait value and maximal buffering of all mutational effects (at the $V_A = 0$ equilibrium) is in general not possible on the phenotype landscape of the multilinear model. Here we see what happens if this constraint is relaxed. In a modularly structured trait, optimization of the trait is still possible even at a point where the marginal effects at some modules vanish. We can think of this as a division of labor among modules; if one module takes on trait optimization, the other modules are "free for other tasks." In the simple additive model, no other tasks exist. In the present model, they use their freedom to evolve to the $V_A = 0$ equilibrium and minimize the genetic variance in their subspace. As a consequence, the evolvable part of the genetic architecture (modules with $V_A \neq 0$) may be much smaller than the entire genetic basis of the trait.

Genetic Effects Measured with Reference to the Population Mean

Whenever genes interact with each other, genetic effects will depend on the genetic background in which they are measured. In the multilinear model, this dependency is incorporated by having model variables and parameters explicitly defined in relation to a particular reference genotype. Several choices of reference genotype are possible. In this article, we have so far used a fixed optimal genotype as our reference. This means that all reference-independent population level observables (such as $\bar{x}$, V_G, V_m) are expressed in terms of reference-dependent variables (ε_{ij}, V_i) that are measured in this genotype. This is adequate if we want to describe evolution on the phenotype landscape in a fixed coordinate system. However, if we were to relate these results to data on locus variances and epistatic interactions, it must be remembered that these effects are not necessarily estimated in an optimal background. In order to make this connection, it is helpful to express the results with reference to the mean values themselves.

We will also see that some of the reference-dependent quantities, namely the effective epistasis coefficients from equations (37), attain a meaning on the population level if measured in this special reference.

General equations for translating from one reference to another are given in Hansen and Wagner (2001*b*; see also eqq. [6] above), but here we will only provide some select observations. One important thing to note is that, under linkage equilibrium, the average epistasis factors, $\bar{f}_i$, are all equal to 1 in the equilibrium reference. This is because they are linear functions of the reference effects, y_j, which necessarily have mean 0 when measured relative to their own mean. Using this and the transformation rules $\hat{\varepsilon}_{ij} = \varepsilon_{ij}/(\bar{f}_i\bar{f}_j)$, $\hat{V}_i = \bar{f}_i^{\,2} V_i$, and $\hat{\mathcal{F}}_i^{\varepsilon} = \mathcal{F}_i^{\varepsilon}/\bar{f}_i$, where the variables in the mean reference are denoted by a "hat," the effective epistasis coefficients from equations (37) in this reference read

$$\hat{\varepsilon}_i = \frac{1}{V_A}\sum_{j\neq i}\hat{\varepsilon}_{ij}\hat{V}_j = \frac{1}{V_A}\sum_{j\neq i}\frac{\varepsilon_{ij}}{\bar{f}_i\bar{f}_j}V_j\bar{f}_j^{\,2} = -\frac{1}{2sV_A}\hat{\mathcal{F}}_i^{\varepsilon}, \quad (46)$$

$$\hat{\Theta}^2 = \frac{1}{V_A^2}\sum_{i,j\neq i}\hat{\varepsilon}_{ij}^2\hat{V}_i\hat{V}_j = \frac{1}{V_A^2}\sum_{i,j\neq i}\varepsilon_{ij}^2V_iV_j = \frac{2}{V_A^2}V_{AA}. \quad (47)$$

This shows how the effective epistasis coefficients provide a link between the functional (Hansen and Wagner 2001*b*) or physiological (Cheverud and Routman 1995; Phillips et al. 2000) level of epistasis and the population level. Both ε_i and Θ^2 are variance-weighted averages of the functional epistasis coefficients ε_{ij} and depend on the reference. If measured in the mean reference, however, ε_i turns out to be proportional to the directional epistatic selection force $\hat{\mathcal{F}}^{\varepsilon}$. We see that this force is indeed caused by directional epistasis (hence its name). The nondirectional epistasis coefficient Θ^2, on the other hand, is the additive × additive epistatic variance normalized by V_A. Both coefficients have direct consequences on the population-level quantities in equilibrium: The reduction of the genetic variance under house of cards conditions is due to nondirectional epistasis in the mean reference (eq. [34]). The deviation of the mean phenotype from the optimum is caused by directional epistasis as measured in this reference.

The Evolution of the Genetic Architecture

In this section, we discuss how mutation and selection shape the genetic architecture of the trait as the population

evolves on the epistatic phenotype landscape. Clearly, any evolution of locus effects is only possible with epistasis. We therefore start out with an analysis of the strength of directional epistasis.

The Strength of Epistatic Selection

Directional epistatic selection measured by $\mathcal{F}^{\varepsilon}$ is important for many results derived in this article. In order to estimate its strength, we now determine the selection coefficients for single mutations due to the epistatic force alone. Consider a mutation of size $-\delta_i$ ($\delta_i > 0$), as measured in the reference genotype at the ith locus. If δ_i is not too large, the selection coefficient is given by the product of $-\delta_i$ and the corresponding entry of the epistatic force vector

$$s_{\varepsilon,i} := s\frac{\partial}{\partial \bar{y}_i} V_A \delta_i = 2sV_A \hat{\varepsilon}_i \hat{\delta}_i. \tag{48}$$

Here, $\hat{\varepsilon}_i$ is the effective directional epistasis coefficient measured relative to the mean genotype given in equation (46), and $\hat{\delta} = \bar{f}_i \delta_i$ is the mean phenotypic effect of the mutation. The dimensionless quantity $\hat{\varepsilon}_i \hat{\delta}_i$ then measures the average directional epistatic effect of the mutation δ_i on the alleles that segregate in the population ($|\hat{\varepsilon}_i \hat{\delta}_i| = 0.1$, meaning that the average allelic effect relative to the population mean is changed by 10%).

The selection coefficient $s_{\varepsilon,i}$ should be compared with the coefficient $s_{a,i} := 2s\bar{x}\hat{\delta}_i$ for directional selection due to the force $\mathcal{F}^{a}$, which is proportional to the distance from the optimum. The size of $s_{\varepsilon,i}$ depends on the strength of directional epistasis $\hat{\varepsilon}_i$ and on V_A. In the HC regime, the additive genetic variance is, to leading order, proportional to the trait mutation rate, $V_A \approx U/s$, and we obtain $s_{\varepsilon,i} \approx 2U\hat{\varepsilon}_i \hat{\delta}_i$. This parallels the result of Wagner et al. (1997) for selection coefficients in a model for the evolution of canalization; that is, under HC conditions, selection coefficients for mutations that alter the genetic architecture of a trait are proportional to the character mutation rate and, in particular, independent of the strength of selection s. For standard values of character mutation rates (U of the order 10^{-2} to 10^{-1} and higher for life-history traits [Lynch et al. 1999]) and not too strong epistasis ($|\hat{\varepsilon}_i \hat{\delta}_i| < 0.1$), we obtain selection coefficients $s_{\varepsilon,i}$ up to 10^{-2} but probably more likely of the order 10^{-3} or 10^{-4}. Selection in this range is moderate to weak. In contrast to the evolution of dominance, where the entire selective advantage is due to reduction of mutational effects at a single locus (cf. Mayo and Bürger 1997), directional epistatic selection results from the joint action of a larger number of genes. This leads to selection coefficients on an intermediate level, lower than the character mutation rates but most likely significantly larger than mutation rates of single loci. We predict in this article that directional epistasis will be small at equilibria (see "The Evolution of Epistatic Interactions" below). This, however, should not be taken as evidence that epistatic selection is weak in general. On the contrary, using equilibrium values for $\hat{\varepsilon}_i$ in equation (48) may severely underestimate the role of epistatic selection for the evolution to the equilibrium.

In the above estimate, we have assumed that genetic variance at the various loci is maintained solely by mutation-selection balance in the HC regime. If other mechanisms contribute to this variation, larger selection coefficients are possible. Note, in particular, that the force $\mathcal{F}^{\varepsilon}$ acts, of course, also away from equilibrium. Fluctuating selection strength (hard winters) could be a potent mechanism to increase selection coefficients. A 10-fold increase of the selection strength leads to a 10-fold increase of the selection coefficient for a few generations because the amount of genetic variance is not immediately in mutation-selection equilibrium. Higher selection coefficients are expected as long as the variances have not yet approached their new smaller equilibrium value. Note that this situation is different from a fluctuating selection optimum, which can also favor the evolution of reduced mutational effects in certain situations (Kawecki 2000) with selection coefficients in a similar range as estimated above. A scenario where much larger selection coefficients can be found is when part of the genetic variation is due to gene flow in a spatially structured population (J. Hermisson and G. P. Wagner, unpublished manuscript).

The Evolution of Mutational Effects

A conspicuous phenomenon that we have observed for the two-locus model is that, in equilibrium, loci with different mutation rates exhibit large differences in their variances of mutational effects, with the locus with the higher mutation rate evolving the smaller variance. We will now argue that this is in fact a general phenomenon of the equilibrium genetic architecture of a polygenic trait.

As indicated above, it is the directional epistatic force $\mathcal{F}^{\varepsilon}$ (eq. 19) that drives the evolution of the genetic architecture. Since $\mathcal{F}^{\varepsilon}$ points into the direction of steepest descent of the additive genetic variance, it drags the population into flatter regions of the phenotype landscape. For a more detailed discussion of the consequences of directional epistasis, it is advantageous to partition this force into components that correspond to the various loci; that is, $\mathcal{F}^{\varepsilon} = \sum_i \mathcal{F}^{\varepsilon,i}$. If $V_{A,i} = \bar{f}_i^{\,2} V_i$ and $V_{AA,i} = (1/2)\sum_j \varepsilon_{ij}^2 V_i V_j$ are the contributions of the ith locus to the additive and additive × additive variance, respectively, we obtain

$$\mathcal{F}^{\varepsilon,i} = -s\nabla_{\bar{y}} V_{\mathrm{A},i} = -s\big(V_{\mathrm{A},i} + 2V_{\mathrm{AA},i}\big)\frac{\nabla_{\bar{y}} V_{\mathrm{m},i}}{V_{\mathrm{m},i}}. \quad (49)$$

Force $\mathcal{F}^{\varepsilon,i}$ points into the direction of steepest descent of the mean locus mutational variance $V_{\mathrm{m},i} = u_i\langle f_i^2\rangle\gamma_i^2$. Two observations are of importance. First, a larger locus contribution to the additive and the additive × additive genetic variance leads to a stronger reduction of the mutational variance on this locus. In (or near) mutation-selection balance, $V_{\mathrm{A},i} + 2V_{\mathrm{AA},i}$ is positively correlated with the locus mutation rate. In particular under HC conditions, $V_{\mathrm{A},i} + 2V_{\mathrm{AA},i} = u_i/s$ (cf. eq. [17]). Selection for reduced mutational variance will be stronger at loci with high u_i. Second, selection for reduced mutational variance does not necessarily subside if $V_{\mathrm{m},i}$ is already small. The reason is that it is the relative, and not the absolute, change that enters the equation.

From this analysis, we predict that our observations in the two-locus model hold true also in the general multilocus case. Selection due to directional epistasis will, on average, create a negative correlation between the locus mutation rates, u_i, and the mean variance of the mutational effects, $\langle f_i^2\rangle\gamma_i^2$, at the same locus.

The Evolution of the Mutational Variance

In the previous subsection, we discussed the consequences of epistatic selection on the mutation effects of single loci. Let us now consider its impact at the level of the trait. Since directional epistasis works to reduce the mutational variance for each locus, it would be natural to also expect that the mutational variance V_{m} of the trait is reduced, meaning that epistasis leads to the evolution of genetic canalization (Waddington 1957). Indeed, the $V_{\mathrm{A}} = 0$ equilibrium, where the marginal effect of all mutations vanishes (complete canalization), would be the unique and globally stable equilibrium if the evolutionary dynamics were driven by $\mathcal{F}^{\varepsilon}$ alone. Surprisingly, however, a reduction of V_{m} is generally not found under the full dynamics, contra previous predictions (Wagner et al. 1997; Rice 1998). On the contrary, our results frequently show a marked increase of V_{m}. Let us illustrate this first with a simple example.

At the mixed-regimes equilibrium of the two-locus model with $s = \gamma_{1,2}^2 = 0.05$ and $u_1 = u_2/2 = 0.0001$, the mutational variance according to equation (28) is $V_{\mathrm{m}} = 2.502 \times 10^{-4}$ and is almost entirely due to the locus with the smaller mutation rate u_1. This value is much higher than the very low $V_{\mathrm{m}} \approx 6 \times 10^{-7}$ at the unstable $V_{\mathrm{A}} = 0$ equilibrium (for moderate epistasis $\varepsilon \approx 1$). It is also more than 17-fold the minimal V_{m} on the contour of all genotypes that lead to the same phenotype as at the equilibrium point or the minimum on the contour of the optimal phenotype (which are both $V_{\mathrm{m}} \approx 1.4 \times 10^{-5}$). We see that the high equilibrium V_{m} is not simply a consequence of the fitness constraint that keeps the mean phenotype close to the optimum.

The reason for this unexpected result is that, in contrast to the locus level (see eq. [49]), the gradients of the (additive) *genetic* variance ∇V_{A} and of the *mutational* variance ∇V_{m} are usually not collinear on the level of the trait. Since the selection force $\mathcal{F}^{\varepsilon}$ is parallel to $-\nabla V_{\mathrm{A}}$ (and to $-\nabla V_{\mathrm{G}}$; see eq. [19]) but in general not to $-\nabla V_{\mathrm{m}}$, the reduction of V_{A}, but not of V_{m}, is the target of epistatic selection on the trait level.

Here, reduction of V_{A} being a target of selection means that V_{A} is reduced as far as possible, given the adaptive constraint that keeps the population mean close to the optimum. This can be seen as follows. At a balancing-forces equilibrium, $\mathcal{F}^{\varepsilon} = -\mathcal{F}^{\mathrm{a}}$, hence ∇V_{A} is collinear to the gradient of the phenotype landscape, $\nabla V_{\mathrm{A}} = -2\bar{x}\nabla\bar{x}$, or perpendicular to the isophenotype contours. As pointed out by Rice (1998), this implies that the genetic variance at a stable equilibrium is at a local minimum with respect to variations of the locus means on the contour.

On the other hand, although $\mathcal{F}^{\varepsilon}$ always points into a direction of reduced V_{m}, it is, in general, not parallel to its gradient. Equilibria therefore do not have to be (and generally are not) minima of V_{m} on the isophenotype contour. As a by-product of the reduction of V_{A}, evolution of the population along the contour often leads to an increase of V_{m}. In figure 3, we show how the evolution of the locus variances even reinforces the divergence of the gradients of V_{A} and V_{m}.

Neither an increase nor a decrease of V_{m} is a necessary consequence of epistasis and stabilizing selection. For two loci under Gaussian conditions, the mutational variance in equilibrium is indeed at a minimum on the isophenotype contour: since $\gamma_1^2 u_1/\gamma_2^2 u_2 = V_1/V_2$, the gradients of V_{A} and of V_{m} are collinear at this point. This, however, seems to be a coincidental property of the two-locus model rather then a consequence of the Gaussian regime. A similar relation $\gamma_i^2 u_i \propto V_i$ does not hold generally for three or more loci. We therefore do not expect that, in equilibrium, V_{m} is at a minimum in these cases.

We thus find that evolution on an epistatic landscape typically leads to a reduction of the additive genetic variance but also in many cases to a marked increase of the mutational variance. High values of V_{m} and low values of $V_{\mathrm{A}}/V_{\mathrm{m}}$ are consistent with the experimental data for many traits (Houle et al. 1996). From the classical view that stabilizing selection entails canalization (i.e., reduces V_{m}), this result is, however, quite unexpected.

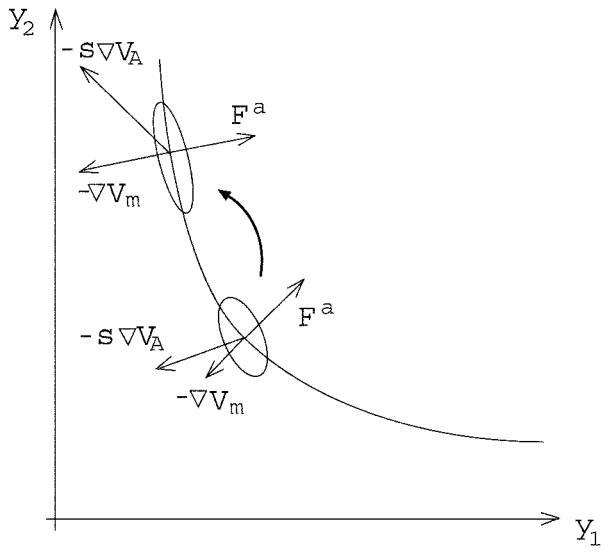

Fig. 3: Evolution along an isophenotype contour in the two-locus model with $\sigma^2_{m,1} = \sigma^2_{m,2}$, but $u_2 > u_1$ under house of cards conditions. We compare $-\nabla V_m \propto -(\bar{f}_1, \bar{f}_2)$ and $\mathcal{F}^e = -s\nabla V_A \propto -(V_1\bar{f}_1, V_2\bar{f}_2)$. Suppose the population is initially at the minimum of V_m on the contour where $\bar{f}_1 = \bar{f}_2$. Since $u_2 > u_1$, $-\nabla V_A$ is at an angle to $-\nabla V_m$, and the means will start to move along the contour, increasing V_m. In the new genetic background, the selection pressure at both loci is changed, leading to a decrease of V_1 and to an increase of V_2. This change in the locus variances changes the direction of ∇V_A relative to ∇V_m, increasing the angle between the two vectors. The gradients of V_A and V_m are driven farther and farther apart.

The Evolution of Epistatic Interactions

As the final aspect of the genetic architecture considered in this article, let us now discuss the evolution of epistatic interactions as measured by the effective directional epistasis coefficient $\hat{\varepsilon}_i$ in the mean reference (see eq. [46]). By considering how this quantity is shaped under natural selection, we get a better sense of what to expect when we observe directional epistasis in a real population.

In equation (46) we have seen that $\hat{\varepsilon}_i$ is proportional to the ith component of the epistatic selection force $\mathcal{F}^e$. Using this in the eigenvalue equation (23) leads to

$$\hat{\varepsilon}_i = -\bar{x}/V_A \quad \forall i, \tag{50}$$

where $\bar{x}$ is the deviance of the trait mean from the optimum. Thus, it is generally true that directional epistasis should be equally strong for all loci when measured in the equilibrium and that the effective epistasis coefficients should equal (minus) the deviation of the trait mean from the optimum divided by the additive genetic variance. One important consequence of this result is that directional epistasis can only arise in equilibrium when the mean is shifted from the optimum. Thus, we may expect epistasis to be largely nondirectional when measured on a well-adapted trait in a balance between symmetric mutation and stabilizing selection.

This result can give us insights into the evolution of genetic architecture. Imagine that there are two independent sets of internally interacting loci as in the modular model above. Assume further that all the loci in one module have only positive effects on each other, while those in the other module have only negative effects on each other. This means that the effective epistasis coefficients will be positive for all loci in the first module but negative for all loci in the second module. This contradicts equation (50), and this situation is thus impossible. What does this mean? It means that this is an example of a genetic architecture that cannot evolve in mutation-selection balance. Imagine we start with this type of genetic architecture defined with reference to any specific genotype, for example, an optimum genotype, and let the system evolve into mutation-selection balance. Then the epistasis factors will evolve to be such that, if we choose to measure the parameters in the equilibrium, they will reveal a totally different pattern of interactions.

Thus, the patterns of gene interactions that we can expect to observe in an equilibrium situation are quite constrained. We have argued above and in appendix B that the deviation of the mean trait from the optimum may be very small in many cases. In this case, the predicted absence of directional epistasis in equilibrium may help explain why quantitative genetic models based on constant additive genetic variation (e.g., Lande 1976*b*, 1979) seem to provide a good prediction for the response to selection over one or a few generations. Only directional epistasis (as measured with reference to the mean) will change the response to selection in the trait (Hansen and Wagner 2001*b*).

These results underscore the fact that the observed genetic architecture is a highly evolvable entity that may look utterly different depending on the vantage point from where it is gauged. We note that the predicted absence of directional epistasis in equilibrium is compatible with directional epistasis with reference to any other genotype such as the optimal genotype.

Effects of Epistasis on Genetic Load and Variances

In order to see what changes epistasis brings to the genetic variance and to the mutation load, let us now compare the properties of equilibria in the epistatic model with the well-known properties of an additive trait.

Effects of Epistasis on the Genetic Variance

Epistasis in a phenotypic landscape model plays a dual role that can be called local and global. Both ways, the population genetic quantities are affected. Locally, keeping the locus means fixed at any point of the landscape, epistasis influences the amount of genetic variation that is maintained at the loci in mutation-selection balance. Globally, epistasis gives rise to the epistatic force $\mathcal{F}^{\varepsilon}$, which drags the population across the landscape toward equilibria with characteristic properties. Both effects can be studied separately, and both effects are important for the interpretation of data.

Local effects of epistasis. Assume first that we have empirical estimates for all locus mutation rates u_i and the mean mutational effects $\bar{f}_i\delta_i$. We want to compare the equilibrium values of V_G and V_A in the epistatic model with their estimates in an additive model that uses the same data. In doing so, we keep the locus means $\bar{y}_i$ fixed at their equilibrium values of the epistatic model and analyze the local effects of epistasis (i.e., the local curvature of the landscape) on the variances by replacing the phenotype landscape with a linearized one.

In the HC regime, any amount and pattern of epistasis lead to a reduction of V_G and V_A relative to an additive trait. As shown in equation (34) and in appendix D, the reduction terms are proportional to the components of the epistatic variance V_{A^m} corresponding to the mth order interactions ($V_{A^2} = V_{AA}$). Since $V_{A^m} \propto V_{i_1}V_{i_2}\ldots V_{i_m}$, and $V_i \sim u_i/s$ in the limit $u_i/s \to 0$, these epistatic terms are of second and higher order in this limit. Nevertheless, since the genetic variance of the additive model increases linearly with the number of loci, $U/s = \sum_i u_i/s \propto n$, but $V_{A^m} \propto n^m$, the epistatic contributions can be substantial if the number of loci is high and $U/s \approx 1$. This conclusion assumes, however, that the average size of the epistasis coefficients is kept roughly constant on increasing n. Whether this holds true depends on the structure of the genetic architecture that underlies the trait. If this structure is highly modular, for example, the number of nonzero epistasis coefficients only increases linearly with n.

The Gaussian case is easiest dealt with in the equilibrium reference where all mean epistasis factors $\bar{f}_i$ are equal to 1, and $\langle \hat{f}_i^{\,2} \rangle = 1 + \mathrm{Var}(\hat{f}_i)$. Using this in equations (16) and (8), which are valid in any reference, we obtain

$$V_G = \sum_i \left[1 + \frac{1}{2}\mathrm{Var}(\hat{f}_i)\right]\hat{V}_i = \sum_i \frac{1 + \mathrm{Var}(\hat{f}_i)/2}{\sqrt{1 + \mathrm{Var}(\hat{f}_i)}} v_i \approx \sum_i \left\{1 + \frac{3}{8}[\mathrm{Var}(\hat{f}_i)]^2\right\} v_i, \quad (51)$$

$$V_A = \sum_i \hat{V}_i = \sum_i \frac{1}{\sqrt{1 + \mathrm{Var}(\hat{f}_i)}} v_i, \quad (52)$$

where $v_i = \sigma_{m,i}/\sqrt{s}$ is the locus genetic variance of the additive model. We see that V_G is slightly increased by the local action of epistasis but V_A is decreased. As shown in appendix D, higher-order interactions reduce V_G and V_A.

These results can be understood by distinguishing two effects of local epistasis. On the one hand, epistasis increases the pressure of stabilizing selection on mutations. Let $s(f_i\delta_i)$ be the selection coefficient of a mutation with phenotypic effect $f_i\delta_i$. Then, the mean selection coefficient in the epistatic model will be larger than the selection coefficient of a mutation with the same mean effect in the additive model; that is, $|\langle s(f_i\delta_i)\rangle| > |s(\langle f_i\delta_i\rangle)|$ because of concavity of the fitness function. This explains the reduction of $V_G \propto u/s$ in the HC approximation. On the other hand, epistasis also increases the mutational variance, since $V_{m,i} = \langle f_i^2\rangle\sigma_{m,i}^2 > \bar{f}_i^{\,2}\sigma_{m,i}^2$. Since $V_G \propto \sqrt{V_m/s}$ in the Gaussian approximation, this explains the different result in this case.

The results comparing additive and epistatic models always depend on which quantities are treated as measured and are therefore kept fixed in both models. If, instead of the mean mutational effects, the mutational variances $V_{m,i}$ are kept constant, we find a decrease of V_G due to epistasis also in the Gaussian case. So far, we have also assumed that the mutation parameters are known for the loci separately. Usually, however, this detailed information is not available, and estimates for mutation rates and effects only exist on the level of the trait. In this situation, equal mutational effects at the loci are often assumed simply due to lack of better knowledge.

If we compare the equilibrium genetic variance in the epistatic model with the equilibrium variance of an additive model with the same mutational parameters on the level of the trait, but with identical loci, the effect of epistasis is twofold. The first effect is the local one described above. The second effect is what we call "global": as we have seen above, large differences in the locus mutational effects are typical of the equilibria of the epistatic model.

Global effects of epistasis. The evolution of different locus effects leads to a further reduction of V_G and V_A of the epistatic trait relative to estimates in a uniform additive model for a simple mathematical reason. Numerical and analytical results show that the genetic variance of a single haploid locus under stabilizing selection is in general a concave function of the mutation rate and of the variance of the mutation distribution (Bürger 2000; Waxman and Welch 2003). This is obvious in the Gaussian approximation; in the HC regime, it is a small second-order effect (Bürger 2000, p. 238). For an additive trait in linkage

equilibrium, any variance in u_i or γ_i^2 across loci therefore leads to a reduction of V_G relative to a uniform model with average locus effects and mutation rates (Waxman and Welch 2003).

As an example, consider the two-locus model at the mixed-regimes fixed point with $u_2 > u_1$. According to equations (25) and (28), the additive genetic variance is $V_A = 2(u_1/s - \varepsilon^2 u_1^2/s^2)$. The genetic variance of an additive model with the same single-locus mutation rates and variances (first locus under HC, second under Gaussian conditions) is $V_A = 2u_1/s$. This is less than the equilibrium variance $V_A = (u_1 + u_2)/s$ of an additive model with the same mutational parameters on the trait level but with identical loci. Note that both loci are under HC conditions in this case.

The reduction due to unequal mutational variances is independent of the strength of epistasis, and, in contrast to the local effect described above, it is a first-order effect. Depending on the interaction pattern, substantial reductions of V_A can occur this way. A striking example is the model of a modular trait where the marginal effect (and thus the contribution to V_A) at certain loci may vanish altogether.

Mean Phenotype and Genetic Load at Epistatic Equilibria

For many patterns of epistatic interactions, epistasis exerts a directional selection effect on the population and drags the mean phenotype away from the optimum. This always occurs if there is no redundancy in the interaction patterns among genes, mathematically, if the epistasis matrix **E** is nonsingular. An upper limit for this deviation is the mean phenotype at the $V_A = 0$ equilibrium, $x_{V_A=0}$ (cf. eq. [31]). At the balancing-forces equilibrium, the deviation is given by an eigenvalue of the matrix $-\mathbf{EV}$. It is particularly large and proportional to the number of loci if all pairwise interactions are uniformly positive (or all negative); see equations (38) and (40). In this case, mutations consistently act synergistically in a given direction of the trait and antagonistically (or, diminishing) in the other direction. The trait is then shifted to the antagonistic side. In general, however, stability seems to require that $\bar{x}$ is in fact the smallest eigenvalue of $-\mathbf{EV}$ with the same sign as $x_{V_A=0}$ (cf. app. B). This indicates that the deviation from the optimum will be rather small in many cases.

If we add epistatic interactions to an initially additive trait, the mutation load L is influenced by two opposite factors. Reduction of the genetic variance reduces the load, but deviation of the phenotypic mean from the optimum increases it. Depending on the model and the equilibrium, these factors may cancel (e.g., HC regime for two-locus model with equal mutation rates) or lead to a net reduction of the load. The reduction can be substantial and may drop to half the value without epistasis in extreme cases (trait optimum at a $V_A = 0$ equilibrium under HC conditions). In some rare cases where V_G increases with epistasis (vanishing directional selection in the Gaussian regime; see eq. [44]), we obtain an increase of the load.

Discussion

Modeling the Genetic Architecture of Quantitative Traits

The relation of most quantitative traits to their genetic basis is highly complex and, despite some encouraging progress through quantitative trait loci (QTL) or mutagenesis experiments, still poorly understood (Barton and Turelli 1989; Barton and Keightley 2002). A central question of our study has been how the mutational effect distribution is shaped by natural selection. Answers to this question can be found only if epistatic interactions are accounted for. Incorporating epistasis into a mathematically tractable model, however, is a major problem. Treatment of a multilinear trait with pairwise interactions among loci has revealed pleasant analytical properties. Many central aspects of the theory can be formulated in terms of linear algebra. The equilibrium structure basically follows from the properties of a linear operator given by the product of the matrix of pairwise interactions and the covariance matrix of the locus effects, **EV**. Eigenvalues correspond to the mean phenotype, and eigenvectors determine the average size of mutational effects at the different loci.

Throughout this article, linkage equilibrium has been assumed. While this is a good approximation for an additive trait as long as linkage is not very tight (Bürger 2000, chap. 6), this is less clear if there are epistatic interactions in the model. At least in the Gaussian regime, however, the effects of linkage disequilibria seem to be minor also for multilinear epistasis. As we will show in a separate publication, the framework presented here can be extended to include linkage disequilibria in this case. Deviations from the linkage equilibrium results are found to be second-order corrections in the quasi-linkage equilibrium approximation (J. Hermisson et al., unpublished manuscript).

Epistasis is a complex phenomenon, and a model cannot cover more than a single facet. On the way toward a nonadditive population genetic theory, other and complementary models will be needed for a full picture. There are three special properties of the present approach that restrict its generality. First, the equilibrium distributions in our model are symmetric at all loci. This is changed if mutations or the fitness function are asymmetric or if the trait is no longer multilinear. As may be seen from equation (21), further selection forces may become important

for skewed distributions. Second, another consequence of the multilinearity assumption is that synergistic epistasis among mutations that increase the genotypic value x implies antagonistic interactions among mutations at the same loci that decrease x. This is not necessarily true on a general genotype-phenotype map. Finally, we have assumed that there is ample room for the mutational effect distribution to evolve independently of the genotypic value. The fitness landscape consists of ridges rather than sharp peaks (fig. 1). For rugged landscapes with sharp optima, the population would be constrained to a peak without much potential for the evolution of mutational effects.

Let us finally take a look at the implications of our results for the robustness of conclusions based on the additive, small-effects polygenic models that have dominated the population genetic literature in the past. Here, two assumptions of these models must be distinguished. We have seen that additivity per se may serve as a reasonable first-order approximation in mutation-selection balance as long as $U/s \ll 1$ (U is the character mutation rate) if epistasis is not very strong. For larger U/s, any epistasis (i.e., not just epistasis with a net positive or negative effect) may become important. The assumption of almost equal and small effects, on the other hand, may lead to substantial deviations even for small U/s. This is the case, in particular, if the differences in the locus effects are large enough that some loci are in the Gaussian and some are in the HC regime. From the point of view of the epistatic model considered in this study, and consonant with experimental data, additive approximations should include these potentially large differences and also take the possibility of a negative correlation among locus mutation rates and effects into account.

The Maintenance of Genetic Variation

We have consistently found that epistasis leads to a reduction of the additive genetic variance that can be maintained in mutation-selection balance. In particular, the house of cards approximation for an additive model provides an upper bound for both V_G and V_A. In the Gaussian approximation, there are cases where the total genetic variance may be slightly elevated due to the influence of epistasis. The additive genetic variance predicted from the epistatic model, however, will always be less than the prediction from the additive model with the same single-locus mutational effects. This demonstrates that epistasis cannot be evoked to save the mutation-selection-balance hypothesis when estimated mutation parameters seem insufficient to explain observed levels of additive genetic variation. On the contrary, the ratio of the trait mutation rate and the selection strength must be at least as high as the genetic variance V_G plus the epistatic variance $V_I = V_{AA} + V_{AAA} + \ldots$; that is, $U/s \geq V_G + V_I$ (cf. eq. [34] and app. D).

In natural populations, most quantitative characters show a large amount of additive genetic variation. Whether this variation can be explained by a balance between mutation and selection has been the subject of a long controversy (Lande 1976*a*; Turelli 1984; Barton and Turelli 1989; Houle et al. 1996; Bürger 2000). A central element to this debate is whether the house of cards or the Gaussian approximation for the allelic distributions is more appropriate. For high per-locus mutation rates and weak selection, where the Gaussian approximation is valid, larger values of V_A are predicted. When we find that the reduction in variance due to epistasis is in fact most pronounced in the house of cards case, it thus accentuates the difference between the two models and makes the mutation hypothesis for maintenance of variation even more dependent on the validity of the Gaussian approximation.

On the other hand, our findings on the evolution of the genetic architecture may have some more subtle implications that are favorable to the mutation hypothesis. The tendency for loci with large mutation rates to evolve smaller mutational effects (see "The Mutational Effect Distribution") would lead to a negative correlation between mutation rate on one side, and mutational effects and variances on the other. That a negative correlation between mutation rates and effects exists in nature has been proposed to reconcile a well-known conflict between genic and trait mutation rates (Lynch and Walsh 1998, chap. 12). In a nutshell, the problem is that current estimates of per-locus mutation rate as well as of average effects predict lower additive genetic variance under mutation-selection balance than observed in nature. If, however, as our model suggests, the major loci tend to have on average a lower mutation rate than minor loci, there is a possibility that current mutation rate measurements tend to underestimate the rate of mutation and overestimate the average effect of the mutation. Such an estimation bias would consistently underestimate the amount of genetic variation maintained for a quantitative character under stabilizing selection.

Our general finding of reduced genetic variance in epistatic models stands in marked contrast (but not necessarily in conflict) to the result of Gimelfarb (1989), who found that large amounts of genetic variance can be maintained as balancing polymorphisms for a biallelic epistatic trait under stabilizing selection alone. A similar conclusion has been reached for a biallelic trait under apparent stabilizing selection if there is pleiotropic epistasis for fitness (Gavrilets and de Jong 1993). No study seems to exist for mutation-selection balance in an epistatic biallelic model beyond two loci, but the results for balancing selection

indicate that the behavior may differ from the one observed here for a continuum-of-alleles model, at least for some of the biallelic equilibria. Since the epistasis term used in Gimelfarb (1989) is included as a special case in our model, we can rule out different epistasis patterns as a cause for this discrepancy. The reason seems rather to be the much higher flexibility of a continuum-of-alleles model where more fine tuning of the locus effects is possible and no variation is maintained in the absence of mutation. In order to further clarify this issue, a more detailed study of the equilibrium structure of the biallelic epistatic trait would be needed than is given in Gimelfarb (1989), where only completely polymorphic equilibria up to four loci are studied. For an additive biallelic trait under stabilizing selection, an exhaustive analysis shows that the genetic variation maintained in a typical equilibrium by balancing selection alone sharply declines with the number of loci increasing (Bürger and Gimelfarb 1999).

The Mutational Effect Distribution

An unexpected phenomenon that we observe in this model is that in many cases large differences in the average mutational effects between loci evolve in mutation-selection balance. We also find that loci with higher mutation rates have a bias to evolve smaller mutational effects, and those with smaller mutation rates evolve larger effects. As we have seen above (eq. [49]), this bias results from a higher contribution to the genetic variance of loci with larger mutation rates, which in turn leads to stronger epistatic selection for the reduction of the locus mutational effect.

Genes with large average effect are called major loci, while those with small average effect are called minor loci. The existence of major and minor alleles is a commonly perceived phenomenon in genetics, and it is tempting to speculate that this may be (at least in part) a generic consequence of selection on characters with interacting loci.

While the effects of mutations vary widely, little is known about systematic differences in mutation rates and effects between loci. Mutation-accumulation experiments and single P-element insertions show that mutations with small effects are much more frequent than mutations with large effects, leading to leptokurtic (L-shaped) distributions with high kurtosis (e.g., Mackay et al. 1992; Garcia-Dorado et al. 1999). These experiments, though, conflate the effects of mutations at different loci as well as different mutations at the same locus.

On the one hand, the effect of different alleles of the same locus can be very different. It is known that loci that can have mutations with major effects, like Ubx has on halter morphology, also have alleles with minor effects on the same character (Gibson and Hogness 1996). The QTL often map to the same chromosomal regions as loci with major effects (Lynch and Walsh 1998, chap. 15), and insertions of P-elements in the achete-scute complex lead to small effects on bristle number (Mackay and Langley 1990). Whether this leads to leptokurtic per-locus mutation distributions is still unclear, however. On the other hand, it has been argued that the high kurtosis on the level of the trait is an emergent effect due to unequal locus mutational variances (Welch and Waxman 2002). Systematic loss-of-function mutation of individual loci in yeast indeed shows that there is a wide range of effects among different loci on various traits (Smith et al. 1996). Hence, there seems to be considerable heterogeneity of the effect of mutations among genes as well as among alleles at the same locus. To our knowledge, there is no data relating per-locus mutation rates to the average effect of mutations at the loci. Estimating both the mutation rate and the effect distribution independently is particularly difficult, since the physical mutational target of most genes is not known. Mutations in noncoding, regulatory regions might be responsible for a substantial number of mutations affecting quantitative variation, but the cis-regulatory regions of most genes are not well understood.

The Role of Constraints

The primary selective force that is responsible for the evolution of the genetic architecture in our model, the epistatic force $\mathcal{F}^{\varepsilon}$ (eq. [19]), works to reduce the phenotypic effect of genetic variation contained in the population. In the picture of the phenotype landscape, where differences in height correspond to differences in phenotype (fig. 1), this appears as a trend toward flatter regions of the landscape. In these regions, the phenotypic expression of a given genotypic change is reduced and the phenotype buffered. The epistatic force only subsides if the marginal effect of all alleles, and hence the additive genetic variance, altogether vanishes.

Given that this selective trend toward $V_A = 0$ should also exist in nature, this raises the question why there is usually ample additive variation found in quantitative traits. There are two possible answers to this question. The first possibility is that populations are simply not close to mutation-selection balance.

The second possibility is that physiological and selective constraints prevent the population from eliminating V_A. This scenario is the one suggested by our model where the additive genetic variance at stable equilibria is usually much larger than 0. Two cases can be distinguished. For some patterns of gene interactions (i.e., on some phenotype landscapes), points with $V_A = 0$ simply do not exist (the landscape is nowhere completely flat). This describes the case where V_A cannot evolve to 0 due to pure "physiological" constraints. An extreme example for this is the

usual additive model: if all epistatic coefficients are 0, the genetic architecture is maximally constrained and unevolvable. Usually, a point with $V_A = 0$ does exist in the multilinear model. However, only if this point coincides with an optimal phenotype can trait optimization and complete buffering of the locus effects occur at the same time. In the vast majority of cases, this is not possible (the phenotype landscape is nowhere completely flat on the contour of the optimal phenotype). In these cases, the combination of selective and physiological constraints prevents the population from evolving $V_A = 0$. Only if epistasis is very strong or selection (and thus the selective constraint) is very weak will a stable $V_A = 0$ equilibrium exist.

Since evolution by natural selection needs additive genetic variance as its fuel, equilibria with $V_A = 0$ are evolutionary dead ends, at least until any remaining epistatic variance is turned into additive variance either by drift or an environmental change that itself alters the genetic architecture. As we have seen above, at least some physiological and selective constraints may in fact be necessary for the maintenance of nonzero levels of V_A in mutation-selection balance. In our model, these are constraints between the genotypic value of the trait itself and the mutational variances that can be realized for the contributing loci. This suggests that, while usually thought of as impeding the evolutionary process, certain constraints might actually be needed to maintain evolvability.

These considerations show that constraints are a necessary ingredient for population genetic modeling in any model that allows for the evolution of the mutational effect distribution. The constraint that usually keeps the "optimal" genotypes apart from the maximally buffered ones is an important difference between the class of models studied in this article and the approach in Wagner et al. (1997). Both the universal mapping function (UMF) and modifier models assume that complete buffering of all loci and trait optimization can occur at the same time. The mathematical reason is that genetic changes on the trait and its genetic architecture are represented by different model variables. Biologically, this assumption is not very realistic. But even within the modeling framework itself, this property is nongeneric, as already mentioned in Wagner et al. (1997) and analyzed in detail in Wagner and Mezey (2000). It turns out that the independence of trait values and architecture exists only for the particular genotypic value that is chosen to be the optimal one. It seems impossible to extend these models such that this property is maintained over a wider range of genotypic values (for details, see Wagner and Mezey 2000). These shortcomings were a major motivation for the development of the multilinear model (Hansen and Wagner 2001*b*).

Whereas stable $V_A = 0$ equilibria seem to occur only under very special circumstances in our model, stable equilibria where the marginal allelic effects at some but not all loci vanish are obtained for various patterns of the epistatic interactions. As discussed above for a modular trait, this occurs if the epistatic coupling of loci is partially relaxed, so that there exist groups of quasi-independent loci.

One may ask whether alleles with a vanishing marginal effect in equilibrium but a nonzero effect in a changed genetic background exist in nature. Currently, this question seems difficult to answer. As argued by Templeton (2000), there is a strong bias against detecting alleles or loci of this kind in QTL measurements since usually only chromosomal regions with significant marginal effects are tested for epistasis in the first place. Whereas knowledge at this point is therefore still very limited, some preliminary evidence has been reported in Templeton (2000). Strong epistasis among loci that show no significant marginal effects has been found for viability in *Drosophila mercatorum* (Templeton et al. 1976). Another example is the phenotype coronary artery calcification in humans. Here, no significant marginal effects of the coronary artery disease risk factor Apolipoprotein E have been found in the wild type, although Apolipoprotein E strongly interacts with other heritable coronary artery disease risk factors (Kardia et al. 1999).

Canalization and Genic Buffering

The emergence of some sort of robustness or buffering of the phenotype with respect to environmental or mutational perturbations has long been perceived as one of the main trends in the evolution of the genetic architecture. Triggered by striking experimental observations (reviewed in Scharloo 1991), this idea was first conceptualized as canalization by Waddington (1953, 1957) many years ago. Later, genetic and environmental canalization were distinguished depending on whether the buffered variation is heritable or not (Stearns and Kawecki 1994; Wagner et al. 1997). Genetic canalization can be characterized as reduced mutational variability of phenotype, indicated by a reduction of the mutational variance (Wagner and Altenberg 1996; Wagner et al. 1997; see also Hermisson and Wagner 2003). Already Waddington (1957) proposed the action of stabilizing selection as the ultimate cause of canalization. The idea is quite simple: for a trait under stabilizing selection, any deviation from the optimum reduces fitness. Any mechanism that buffers the trait by decreasing the phenotypic effects of the variations should therefore be favored by natural selection.

As pointed out by Rice (1998), canalization may evolve whenever a phenotype is regulated by two or more gene products that interact in a nonlinear way. A polygenic trait

under stabilizing selection with a continuum of alleles and epistasis among loci is therefore the generic model where evolution of canalization should be expected. Considering this picture, the finding of high levels of mutational variance at many equilibria of the epistatic model is perhaps the biggest surprise of this study. What, now, are the consequences of these results for the concept of canalization?

It should be stressed that the results do not imply that canalization (in the sense of a reduced variability as measured by V_m) cannot evolve at all under stabilizing selection. Indeed, several studies have shown that this is possible (Wagner 1996; Wagner et al. 1997). But first, the conditions under which this occurs may be even more restrictive than previously thought, and second, the converse effect (i.e., an increase of the mutational variance) may equally well occur. It seems, therefore, that the traditional picture of stabilizing selection entailing canalization is no longer tenable from a population genetic point of view. Two factors contribute to this changed view. The first factor is due to the constraints that inhibit simultaneous buffering at all loci; the second one deals with the level of canalizing selection.

As discussed in the previous section, complete canalization, or the complete buffering of mutational effects on the phenotype at all loci, is usually not compatible with trait optimization in the multilinear model. This seems to be a generic property of all but very special phenotype landscapes. Also on the multilinear landscape, however, each phenotype (and, in particular, the optimal one) is represented by a continuum of genotypes that differ in their genetic variability as measured by V_m. In many cases, there is even a most canalized genotype (a "canalization point") with minimal V_m on the contour of the optimal phenotype. The unexpected result now is that V_m is in general not minimized on this more restricted set of genotypes, but it may become rather large. This is in clear contrast to the evolution toward the canalization points predicted in Rice (1998). Note, however, that Rice's prediction was made assuming constant variances at the individual loci or underlying variables. Our result strongly depends on the dynamics of the genetic variance. The reason for this negative result is the incomplete correlation between the additive genetic variance and the mutational variance pointed out in "The Evolution of the Mutational Variance." The reduction of V_m is not itself a target of selection, but the reduction of V_A is.

Nevertheless, the model predicts characteristic patterns of mutational buffering. The level of integration where buffering occurs, however, is different from what Waddington (1957) expected. We do not find canalization of the unit that is exposed to (and constrained by) selection (i.e., the trait). We do, however, find a clear tendency for the evolution of genic canalization, that is, the buffering of the mutational effects of certain subunits or groups of genes. Buffering effects on the genic level are frequently observed in nature (Wilkins 1997; Wagner 2000; Hartman et al. 2001). On the level of a single gene, mutational buffering is indeed coupled with the reduction of V_A (respectively, the locus contribution) in our model, making genic canalization a target of selection (eq. [49]). In the absence of a completely canalized, optimal phenotype, however, the different targets of selection for the buffering of all the genes are in conflict. Here, the model predicts a bias toward buffering of loci with higher mutation rates, often at the cost of decanalization of loci with lower mutation rates. In the sum, decanalization at the latter loci easily overcompensates the buffering effect of the former. Stabilizing selection, therefore, does not necessarily reduce the net impact of new mutations on the trait but may equally well have the opposite effect.

We can summarize these findings in an intuitive picture. Assume a polygenic trait under stabilizing selection in "quasi"-mutation-selection equilibrium (i.e., a population on or near the contour of the optimal phenotype but not yet at the equilibrium point). In this situation, each gene (or each locus) tries to buffer its effect on the phenotype with respect to deleterious effects it might suffer from mutations. In principle, this may be accomplished by restructuring the genetic background accordingly. As it occurs, however, it is not the same background that best serves every gene. Also, buffering efforts are constrained by the requirement that the mean trait value must stay near the optimum. In order to resolve this conflict, the genes cast their ballot in the parliament of genes on every mutation δ_j that changes the mutational effects. There is, however, no "one gene, one vote" principle in force in this parliament. The votes are weighted according to relative buffering effects ($\triangle_{y_j} V_{m,i}/V_{m,i}$) of the mutation and according to the respective contributions of the loci to the genetic variance on the trait ($\propto V_{A,i} + 2V_{AA,i}$); see equation (49). Under house of cards conditions, this contribution is proportional to the gene mutation rate u_i. Clearly, this makes the genes with the higher mutation rates the "more equal" members of parliament in this game that will achieve, on average, higher buffering. The selection coefficient for the buffering mutation corresponds to the margin of the vote. For many mutations this margin will be much higher than just one vote, leading to selection coefficients that are considerably higher than in the case of selection for dominance, which is a single-locus buffering problem. Let us finally consider how the "common good" is served by this procedure. If buffering on the level of the trait (low mutational variance) measures the common good, we have seen that this quantity is in general not maximized. In contrast, it is quite often driven to low values. The reason for this phenomenon is, of course, that

the majority can (and will, for selfish genes) assert even small advantages at a high cost for the minority.

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APPENDIX A

Calculations for Two Loci

From the eigenvalue equation (23) and the relations (7) and (8) for the mean and the additive variance, we derive $\bar{x} = -\varepsilon(V_1V_2)^{1/2}$ and $V_A = 2(V_1V_2)^{1/2} - 2\varepsilon^2V_1V_2$. For the equilibrium to exist, $V_A \geq 0$ must hold, and this leads to the condition $\varepsilon^2(V_1V_2)^{1/2} < 1$ and also excludes the solution $\bar{x} = +\varepsilon(V_1V_2)^{1/2}$ of the eigenvalue equation. The epistasis factors are related to the variances as $\langle f_1^2\rangle = \bar{f}_1^{\,2} + \varepsilon^2V_2 = (V_2/V_1)^{1/2} = \langle f_2^2\rangle^{-1}$.

Under Gaussian conditions, we now obtain $V_1 = (\sigma^3_{m,1}/s\sigma_{m,2})^{1/2}$ and $V_2 = (\sigma^3_{m,2}/s\sigma_{m,1})^{1/2}$ for the variances of reference effects at the two loci. Using these, the population means and variances follow from the relations (eqq. [7]–[12]). In the house of cards regime, inserting the above expressions for $\langle f^2_{1,2}\rangle$ into equation (17) gives $(V_1V_2)^{1/2} = u_1/s = u_2/s$, which shows that the locus mutation rates must coincide. In this case, we have $\langle f_1^2\rangle V_1 = V_2/\langle f_1^2\rangle = u/s$ for the variation of genetic reference effects at the loci, where $\langle f_1^2\rangle$ parameterizes the contour of equilibrium solutions. In the mixed regime, with the first locus under HC conditions, we can still repeat the above calculations to obtain $(V_1V_2)^{1/2} = u_1/s$. Equating the expression $V_2 = \langle f_1^2\rangle u_1/s$ for the genetic variance at the second locus with the solution of the Gaussian equilibrium condition on this contour, $V_2 = (\langle f_1^2\rangle/s)^{1/2}\sigma_{m,2}$, fixes the parameter $\langle f_1^2\rangle$ to $\langle f_1^2\rangle = s\gamma_2^2u_2/u_1$.

APPENDIX B

Stability of Equilibria

In this appendix, we determine the stability of the equilibria derived above. As for the solutions themselves, this is only possible approximately. We assume that linkage equilibrium, the symmetry of the distribution, and the Gaussian or house of cards conditions are maintained under perturbations. Stability is not checked with respect to arbitrary perturbations but only with respect to changes in the means and the variances. This restricts the analysis to a subset of distributions in a $2n$-dimensional vector space. In this vector space, asymptotic (local) stability is determined by the eigenvalues of the Jacobian matrix. Equilibria (or contours of equilibria) are classified as stable if and only if the real parts of all nonzero eigenvalues are negative and the eigenvectors corresponding to the zero eigenvalues are tangent vectors of the contour of equilibrium solutions. The entries of the Jacobian matrix are (using $\varepsilon_{ii} \equiv 0$)

$$\frac{\partial\Delta\bar{y}_i}{\partial\bar{y}_j} = -2sV_i\Big(\bar{f}_i\bar{f}_j + \varepsilon_{ij}\bar{x} + \sum_{k\neq i,j}\varepsilon_{ik}\varepsilon_{jk}V_k\Big),$$

$$\frac{\partial\Delta\bar{y}_i}{\partial V_j} = -2sV_i\varepsilon_{ij}\bar{f}_j. \tag{B1}$$

For house of cards, we further obtain

$$\frac{\partial\Delta V_i}{\partial\bar{y}_j} = -2s\gamma_i^2V_i\bar{f}_i\varepsilon_{ij},$$

$$\frac{\partial\Delta V_i}{\partial V_i} = -s\gamma_i^2\Big(\bar{f}_i^{\,2} + \sum_{i,j\neq i}\varepsilon_{ij}^2V_j\Big), \tag{B2}$$

$$\frac{\partial\Delta V_i}{\partial V_j} = -s\gamma_i^2\varepsilon_{ij}^2V_i.$$

The corresponding expressions under Gaussian conditions are

$$\frac{\partial\Delta V_i}{\partial\bar{y}_j} = -2sV_i^2\bar{f}_i\varepsilon_{ij},$$

$$\frac{\partial\Delta V_i}{\partial V_i} = -2sV_i\Big(\bar{f}_i^{\,2} + \sum_{i,j\neq i}\varepsilon_{ij}^2V_j\Big), \tag{B3}$$

$$\frac{\partial\Delta V_i}{\partial V_j} = -sV_i^2\varepsilon_{ij}^2.$$

The spectrum of the Jacobian matrix has been analyzed analytically or numerically for all equilibria described in the article. For the $V_A = 0$ equilibria in the two-locus model, the condition $\varepsilon^2(V_1V_2)^{1/2} > 1$ for stability is easily derived. For the balancing-forces equilibrium, we obtain the following results:

House of cards. There is a 0 eigenvalue with corresponding eigenvector in the direction of the equilibrium contour. For $s \geq \varepsilon^2u$ (which is needed for the existence of the equilibrium), and under house of cards condition ($\gamma_i^2 \geq V_i$), the real parts of the three other eigenvalues are negative. This follows from an analytical analysis of the discriminant of the characteristic polynomial.

Gaussian. Defining $G := \varepsilon^2(\sigma_{m,1}\sigma_{m,2}/s)^{1/2}$, the four eigenvalues are given by $-(sG/2\varepsilon^2)[6 - 3G \pm (4 - 4G + 9G^2)^{1/2}]$ and $-(sG/2\varepsilon^2)[2 + 3G \pm (4 - 4G + 9G^2)^{1/2}]$. The equilibrium is stable whenever it exists, that is, for $0 \leq G \leq 1$.

Mixed regimes. The spectrum depends on three independent variables, γ_1^2, γ_2^2, and $\hat{\varepsilon} = \varepsilon^2 u/s$. Numerical evidence shows that the equilibria are stable whenever they exist.

For the four-locus model with two submodules (app. C, eq. [C1]), numerical results indicate that the equilibrium with $\bar{f}_i \neq 0$, $\forall i$ is always unstable. The results for the other equilibria follow from the two-locus results after appropriate changes of parameters.

In order to obtain hints for the stability of equilibria also in the general multilocus case, it is instructive to restrict the stability analysis even further to perturbations in the locus means alone. The entries of the corresponding n-dimensional Jacobian matrix are given by the first expression in equations (B1). We can write this matrix as $-2s\mathbf{Q}$, where $\mathbf{Q} = (\mathbf{VE})^2 + \mathbf{VE}\bar{x} + \mathbf{F}$ with $F_{ij} = V_i\bar{f}_i\bar{f}_j$. The right eigenvectors of $\mathbf{Q}$ are the left eigenvectors of $\mathbf{EV}$. Its spectrum is $\{\lambda_i^2 + \lambda_i\bar{x} + V_A\delta_{\lambda_i+\bar{x}}\}$, where λ_i is taken from the eigenvalues of $\mathbf{EV}$ and $\delta_{\lambda_i+\bar{x}} = 1$, if $\lambda_i + \bar{x} = 0$ and 0 else. According to the restricted dynamics, the equilibrium is therefore stable (all eigenvalues of $\mathbf{Q}$ are positive) if $\bar{x}$ is smaller or equal in absolute value than the smallest eigenvalue of $-\mathbf{EV}$ with the same sign. For the $V_A = 0$ equilibrium where the dynamics of the means and the variances decouple (which may be seen by setting $\bar{f}_i = 0$ in eqq. B1–B3), this translates into a necessary condition for the stability of the equilibrium in the above framework. If we decrease the strength of epistatic interactions in a system with a stable $V_A = 0$ equilibrium, this equilibrium becomes unstable as soon as the smallest eigenvalue of $-\mathbf{EV}$ crosses $x_{V_A=0}$. This is precisely when the first balancing-forces equilibrium appears. For balancing-forces equilibria, the argument seems to indicate that the deviation from the optimum is always given by the smallest eigenvalue of $-\mathbf{EV}$ (of the same sign as $x_{V_A=0}$).

APPENDIX C

Multilocus Solutions

Solutions for Singular Epistasis Matrix

For all equilibria with reference effects $|\bar{y}_i| < \infty$ at all loci, the vector of mean epistasis factors $\bar{\boldsymbol{f}} = \mathbf{1} + \mathbf{E}\bar{\boldsymbol{y}}$ must solve the eigenvalue equation of the operator $\mathbf{EV}$. Here we show that in the case of an epistasis matrix $\mathbf{E}$, with (at least) one eigenvalue 0, one of the following cases applies:

If the vector $\mathbf{1} = (1, 1, 1, \ldots)$ is in the image of $\mathbf{E}$, a solution with $\bar{\boldsymbol{f}} = \mathbf{0}$ exists, but no solution with $\bar{x} = 0$ as eigenvalue of $\mathbf{EV}$.

Otherwise, if $\mathbf{1}$ is not in the image of $\mathbf{E}$, a solution with $\bar{x} = 0$ exists, but no solution with $\bar{\boldsymbol{f}} = \mathbf{0}$.

The part for $\bar{\boldsymbol{f}} = \mathbf{0}$, which defines the $V_A = 0$ equilibrium, directly follows from $\bar{\boldsymbol{f}} = \mathbf{1} + \mathbf{E}\bar{\boldsymbol{y}}$. For a solution with $\bar{x} = 0$, $\mathbf{E}$ must have an eigenvalue 0 with eigenvector $\mathbf{V}\bar{\boldsymbol{f}}$. Then, $\bar{\boldsymbol{f}}^t\mathbf{V}\bar{\boldsymbol{f}} = \bar{\boldsymbol{f}}^t\mathbf{V}\mathbf{1} + \bar{\boldsymbol{f}}^t\mathbf{V}\mathbf{E}\bar{\boldsymbol{y}} = \bar{\boldsymbol{f}}^t\mathbf{V}\mathbf{1}$. If $\mathbf{1}$ is in the image of $\mathbf{E}$, this expression is 0 since the kernel and the image of a symmetric matrix are orthogonal. In this case, however, necessarily $\bar{\boldsymbol{f}} = \mathbf{0}$ since $\mathbf{V}$ is invertible. Otherwise, if $\mathbf{1}$ is not in the image of $\mathbf{E}$, the vector space of all epistasis factors, which is spanned by $\mathbf{1}$ and the image of $\mathbf{E}$, has a nontrivial intersection with the kernel of $\mathbf{E}$. Since $\mathbf{V}$ is invertible, the same holds true for the vector space of vectors $\mathbf{V}\bar{\boldsymbol{f}}$, which proves the assertion.

A Four-Locus Model

Consider a trait that is determined by two modules with two loci each:

$$x = y_1 + y_2 + y_3 + y_4 + \varepsilon_{12}y_1y_2 + \varepsilon_{34}y_3y_4. \qquad \text{(C1)}$$

Analyzing the eigenvalue equation (23) for this model as described in the modular-trait section above we find that four equilibria can exist. There is an equilibrium with both modules at the balancing-forces equilibrium in their subspace; that is, $\bar{f}_i \neq 0$, $\forall i$ (result not shown). A numerical stability analysis indicates, however, that this equilibrium is unstable whenever it exists. In the other three cases, the marginal effects of all mutations vanish in the subspace of at least one of the modules. As it turns out, the equilibrium structure of the other module coincides with that of the two-locus model (treated above) after a transformation of the parameters. If the second module is kept at the $V_A = 0$ equilibrium, that is, $\bar{f}_{3,4} = 0$, the background effect of this module on the first module consists of an effective shift of the optimal trait value to $x_{V_A=0} = 1/\varepsilon_{34}$. In figure 2, this leads to the following changes of parameters:

$$\varepsilon \to \tilde{\varepsilon} = \frac{\varepsilon_{12}\varepsilon_{34}}{\varepsilon_{12} + \varepsilon_{34}},$$

$$\gamma_{1,2} \to \gamma_{1,2}\sqrt{\frac{\varepsilon_{12}}{\tilde{\varepsilon}}}, \qquad \text{(C2)}$$

where $\tilde{\varepsilon}$ is an effective epistasis parameter that depends on the epistasis coefficients in both modules. With these rescaled parameters, all means and variances are obtained

from the two-locus results: the mean phenotype is the one from the first subsystem (under the above rescaling), the genetic and mutational variances are given by the sum of the respective quantities in both subsystems, and the load follows from these values. (Note that the γ_i contained in the $\sigma_{\mathrm{m},i}$ must also be rescaled.) If the first module is in the mixed regime (with $u_2 > u_1$) and the second module is under Gaussian conditions, we obtain, for example,

$$\begin{aligned}\bar{x} &= -\tilde{\varepsilon}\frac{u_1}{s},\\ V_{\mathrm{G}} &= 2\frac{u_1}{s} - \tilde{\varepsilon}^2\frac{u_1^2}{s^2} + \left(\frac{\varepsilon_{34}\sigma_{\mathrm{m},3}\sigma_{\mathrm{m},4}}{s}\right)^{2/3},\\ L &= 2u_1 + \left(s\varepsilon_{34}^2\sigma_{\mathrm{m},3}^2\sigma_{\mathrm{m},4}^2\right)^{1/3}\\ V_{\mathrm{m}} &= s\left(\gamma_1^2\gamma_2^2\frac{\varepsilon_{12}^2u_2}{\tilde{\varepsilon}^2u_1} + \frac{u_1^2}{s^2}\right) + 2\left(\frac{\varepsilon_{34}^4\sigma_{\mathrm{m},3}^4\sigma_{\mathrm{m},4}^4}{s}\right)^{1/3}.\end{aligned} \tag{C3, C4}$$

There is a substantial reduction (linear in the mutation rate) of the genetic load and the genetic variances relative to the additive model. Depending on the relative size and sign of ε_{12} and ε_{34}, the mutational variance, on the other hand, may be significantly increased.

Since an equivalent result holds with both modules exchanged, we see in particular that two stable equilibria with either module at the $V_{\mathrm{A}} = 0$ equilibrium in its subspace exist for a large parameter range.

APPENDIX D

Higher-Order Epistatic Interactions

In this appendix, we expand some of our results on the effects of epistasis on the genetic variance in mutation-selection balance to higher-order interactions. That is, we now consider the full multilinear model for the trait,

$$x = \sum_i y_i + \sum_{i<j}\varepsilon_{ij}y_iy_j + \sum_{i<j<k}\varepsilon_{ijk}y_iy_jy_k + \dots . \tag{D1}$$

We consider solutions in mutation-selection balance assuming linkage equilibrium. Since (due to multilinearity) the marginal fitness function on each locus is quadratic and mutation is symmetric by assumption, the equilibrium distribution is symmetric in all y_i. As in the pairwise interaction case, we can set third cumulants to 0 and obtain

$$\Delta V_i = -s\langle f_i^2\rangle\left(C_{4,i} + 2V_i^2\right) + \sigma_{\mathrm{m},i}^2 = 0 \tag{D2}$$

as the equilibrium condition from the dynamical equations of the locus variances. We now expand $\langle f_i^2\rangle$ as

$$\langle f_i^2\rangle = \bar{f}_i^2 + \sum_j\langle\partial f_i/\partial y_j\rangle^2V_j + \sum_{j<k}\langle\partial^2 f_i/\partial y_j\partial y_k\rangle^2V_jV_k + \sum_{j<k<l}\dots . \tag{D3}$$

For a proof of this relation, note first that f_i is a multilinear function in the y_j. In equation (D3), all purely multilinear terms of $\langle f_i^2\rangle$ are contained in $\bar{f}_i^2$. Terms with a quadratic component of the form $\langle y_{j_1}^2y_{j_2}^2\cdots y_{j_{\mathrm{m}}}^2\rangle$ are covered by the term proportional to $V_{j_1}V_{j_2}\cdots V_{j_{\mathrm{m}}}$ on the left-hand side of equation (D3). Finally, all terms proportional to a factor of the form $\bar{y}_{j_1}^2\bar{y}_{j_2}^2\cdots\bar{y}_{j_{\mathrm{m}}}^2$ that are absent from $\langle f_i^2\rangle$ sum up as

$$1 - m + \frac{m(m-1)}{2!} - \frac{m(m-1)(m-2)}{3!} + \dots - \dots = (1-1)^m = 0 \tag{D4}$$

on the left-hand side of equation (D3) and exactly cancel. We express V_{G} as (cf. Hansen and Wagner 2001*b*)

$$\begin{aligned}V_{\mathrm{G}} &= V_{\mathrm{A}} + V_{\mathrm{AA}} + V_{\mathrm{AAA}} + \dots\\ &= \sum_i\bar{f}_i^2V_i + \sum_{i<j}\langle\partial^2x/\partial y_i\partial y_j\rangle^2V_iV_j + \sum_{i<j<k}\langle\partial^3x/\partial y_i\partial y_j\partial y_k\rangle^2V_iV_jV_k + \dots .\end{aligned} \tag{D5}$$

Remembering that $f_i = \partial x/\partial y_i$ and using the house of cards approximation ($C_{4,i} \approx V_i^2\gamma_i^2$) in equation (D2), we obtain

$$V_G = \sum_i \frac{u_i}{s} - V_{AA} - 2V_{AAA} - \ldots - (k-1)V_{A^k} - \ldots, \tag{D6}$$

$$V_A = \sum_i \frac{u_i}{s} - 2V_{AA} - 3V_{AAA} - \ldots - kV_{A^k} - \ldots \tag{D7}$$

under HC conditions. In the Gaussian regime ($C_{4,i} = 0$), we again find that the house of card results hold as an upper limit and that V_A in the epistatic system is always reduced relative to an additive model with the same single-locus mutation rates and effects. The arguments are the same as in the pairwise interaction case. If we keep the locus means fixed and evaluate the Gaussian case in the reference of the mean genotype we obtain

$$V_G = \sum_i \frac{1 + (1/2)\,\mathrm{Var}_1(f_i) + (1/3)\,\mathrm{Var}_2(f_i) + (1/4)\ldots}{\sqrt{1 + \mathrm{Var}(f_i)}} \frac{\sigma_{m,i}}{\sqrt{s}}, \tag{D8}$$

$$V_A = \sum_i \frac{1}{\sqrt{1 + \mathrm{Var}(f_i)}} \frac{\sigma_{m,i}}{\sqrt{s}}, \tag{D9}$$

where $\mathrm{Var}_m(f_i)$ is the mth order contribution to the variance of the epistasis factor f_i, $\mathrm{Var}(f_i)$. It is given by the m-fold sum term in equation (D3). We see that weak second-order epistasis increases V_G while weak higher-order epistasis reduces it. Any order of epistasis reduces V_A.

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