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EVOLUTION OF GENETIC ARCHITECTURE UNDER DIRECTIONAL SELECTION

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Abstract.—We investigate the multilinear epistatic model under mutation-limited directional selection. We confirm previous results that only directional epistasis, in which genes on average reinforce or diminish each other's effects, contribute to the initial evolution of mutational effects. Thus, either canalization or decanalization can occur under directional selection, depending on whether positive or negative epistasis is prevalent. We then focus on the evolution of the epistatic coefficients themselves. In the absence of higher-order epistasis, positive pairwise epistasis will tend to weaken relative to additive effects, while negative pairwise epistasis will tend to become strengthened. Positive third-order epistasis will counteract these effects, while negative third-order epistasis will reinforce them. More generally, gene interactions of all orders have an inherent tendency for negative changes under directional selection, which can only be modified by higher-order directional epistasis. We identify three types of nonadditive quasi-equilibrium architectures that, although not strictly stable, can be maintained for an extended time: (1) nondirectional epistatic architectures; (2) canalized architectures with strong epistasis; and (3) near-additive architectures in which additive effects keep increasing relative to epistasis.

Key words.—Canalization, epistasis, epistatic constraint, evolvability, genetic architecture.

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The most striking fact about natural selection is its immense power in producing refined complex adaptations (e.g., Dawkins 1996). Theoretical work has made it clear that selection acting on typical levels of genetic variation is able to bring a population to a local fitness optimum within a geological blink of an eye (e.g., Fisher 1930; Lande 1976), and there are many empirical examples of rapid evolutionary change that underscores this evolvability (Hendry and Kinnison 1999). There are, however, also many examples of traits that have failed to adapt or seem inexplicably conservative in the light of changing environments (Bradshaw 1991; Williams 1992; Crespi 2000; Gould 2002; Hansen and Houle 2004). It seems clear that both populations and characters within populations differ radically in their evolvability. Despite this, evolvability has only recently become a focus of attention for population-genetics modeling (for review see Hansen 2006).

Because selection acts on variation, evolvability depends on the ability of characters to vary. This propensity to vary has been called ''variability'' and must be distinguished from variation, which refers to the realized differences between individuals (Wagner and Altenberg 1996). Although selection acts directly on the standing variation in the population, it is the variability that determines how much and what type of variation can be made available, and how fast the variation that is lost to selection and drift can be replenished by new mutations. Whereas variation is a fairly ephemeral property of a population, the variability is a property of the individual genotypes. The genotype-phenotype map therefore holds the key to understanding both variability and evolvability.

Most models of evolutionary dynamics explicitly or implicitly assume a very simple genotype-phenotype map, where genes are assumed to act independently of each other and quasi-independently on different characters (Lewontin 1978). In quantitative genetic terms this means that a never-ending supply of unconstrained additive genetic variation can be generated for each character. Although these assumptions are convenient for the modeler and useful in illustrating the power of natural selection, they appear unrealistic on developmental and physiological grounds. Violations of the independence and additivity assumptions are caused by pleiotropy and epistasis. We may therefore discuss pleiotropic and epistatic constraints on evolvability (Hansen and Houle 2004).

Epistasis and pleiotropy may also act as enhancers of evolvability and not just as a constraints. The effects of epistasis on evolvability depends on the directionality of the epistatic interactions. Positive epistatic interactions, in which genetic substitutions reinforce the effects of each other in a given direction in morphospace, will increase evolvability if selection pushes the phenotype in this direction, and negative epistatic interactions, in which genetic substitutions diminish the effects of each other, will have the opposite effect and act as an epistatic constraint (Hansen and Wagner 2001a). This effect was dramatically demonstrated by the individual-based simulations of Carter et al. (2005), who showed that directional selection on standing variation generated by predominantly positive epistatic genotype-phenotype maps led to rapid increases of evolvability and much stronger responses to selection than was found in additive models with comparable parameters. By contrast, predominantly negative epistatic architectures led to rapid loss of evolvability and acted as strong constraints.

To study the evolution of evolvability, we need to study how gene effects, as opposed to gene frequencies, change under selection. The multilinear epistatic model (Hansen and Wagner 2001a,b) was developed for this purpose. The crucial assumption in this model is that the effect of a (set of) gene substitutions is a linear function of changes at other loci. This means that the phenotypic effects of a set of genotypes at a locus can be stretched or compressed relative to each other, but it does not allow any topological changes where the order of the effects are changed (beyond a simple change of sign of all effects). These conditions exclude complex rugged genotype-phenotype maps, but lend themselves to analytical modeling, and allows the evolution of evolvability to happen.

In this paper, we extend the results of Carter et al. (2005) by using the multilinear model to study evolutionary changes in genetic architecture under directional selection and by including mutations to consider evolution on longer time scales.

Model and Background

The Multilinear Model

The multilinear model represents the genotype, g, of an individual as a set of reference effects: $g = \{{}^1y, \ldots, {}^ny\}$, where the reference effect, ${}^iy$, of a locus, i, is defined as the phenotypic effect of substituting the genotype, (i.e., one or two alleles at this locus) into a designated reference genotype. If z is the genotypic value of the individual, the linearity assumption implies that the genotype-phenotype map takes the following form:

$$z = z_r + \sum_i {}^iy + \frac{1}{2!}\sum_i\sum_j {}^{ij}\varepsilon\, {}^iy\, {}^jy + \frac{1}{3!}\sum_i\sum_j\sum_k {}^{ijk}\varepsilon\, {}^iy\, {}^jy\, {}^ky + \cdots, \quad (1)$$

where z_r is the genotypic value of the reference genotype, the ${}^{ij}\varepsilon$ and ${}^{ijk}\varepsilon$ are epistasis coefficients describing the interaction between loci given by the upper left indices (interactions of a locus with itself, such as ${}^{ii}\varepsilon$ or ${}^{iij}\varepsilon$, are set to zero), and summations are over all loci in the set g.

Epistasis factors are descriptors of how a genetic background modifies genetic effects relative to the reference genotype (Wagner et al. 1998). For the multilinear model, the epistasis factor describing the change of a single-locus reference effect is

$${}^{g\to i}f := 1 + \sum_j {}^{ij}\varepsilon\, {}^jy + \frac{1}{2!}\sum_j\sum_k {}^{ijk}\varepsilon\, {}^jy\, {}^ky + \cdots, \quad (2)$$

such that the effect of a particular substitution at locus i with reference effect Δ^iy has effect ${}^{g\to i}f\Delta^iy$ in the background of g. Here, if genotype 1 is substituted for genotype 2 at locus i, we define $\Delta^iy = {}^iy_1 - {}^iy_2$. The epistasis factor describing the change of an interaction among two loci i and j is

$${}^{g\to ij}f := 1 + \sum_k \frac{{}^{ijk}\varepsilon\, {}^ky}{{}^{ij}\varepsilon} + \cdots, \quad (3)$$

such that the effect of substitutions with reference effects Δ^iy and Δ^jy at these loci, which would have an epistatic effect equal to ${}^{ij}\varepsilon\Delta^iy\Delta^jy$ in the reference genotype, will instead have an epistatic effect equal to ${}^{g\to ij}f\,{}^{ij}\varepsilon\Delta^iy\Delta^jy$ in the background of g. Equations for higher-order factors can be found in Appendix 1.

Let the reference effect of an allele r at locus i, be given as ${}^{i(r)}a$, in such a way that the reference effect of a whole-locus genotype with alleles 1 and 2 is given as ${}^iy = {}^{i(1)}a + {}^{i(2)}a$. Thus, we assume that there is no dominance.

Change of Reference as a Tool for Studying Evolution of Genetic Architecture

By definition, epistasis means that the phenotypic effects of gene substitutions depend on the genetic background in which they take place. This necessarily means that the effects will be different if measured in different backgrounds. We therefore explicitly incorporate the reference genotype in which effects are measured into our model. General results may then be derived by use of a set of equations relating parameters measured in one reference to those measured in another. The relationship between parameters measured with reference to two different genotypes, g and h, are

$${}^iy_g = {}^{g\to i}f_h({}^iy_h - {}^id_h), \quad (4a)$$

$${}^{ij}\varepsilon_g = \frac{{}^{g\to ij}f_h\, {}^{ij}\varepsilon_h}{{}^{g\to i}f_h\, {}^{g\to j}f_h}, \quad \text{and} \quad (4b)$$

$${}^{ijk}\varepsilon_g = \frac{{}^{g\to ijk}f_h\, {}^{ijk}\varepsilon_h}{{}^{g\to i}f_h\, {}^{g\to j}f_h\, {}^{g\to k}f_h}, \quad (4c)$$

where the subscripts g and h signify measurement with reference to genotypes g or h, respectively. The parameter ${}^id_h$ is the reference effect of the change at locus i from genotype h to g. These change-of-reference equations, as well as their higher-order analogues, are derived in Hansen and Wagner (2001a). See also Barton and Turelli (2004) for an alternative set of change-of-reference equations for diallelic systems.

The change-of-reference equations provide powerful tools for studying changes in genetic architecture. We start our simulations with all our variables and parameters defined in relation to the mean effects of the starting population. Although the parameters stay constant throughout the simula-

tions, we can study changes in genetic architecture by changing the reference genotype to match the population mean genotype as the simulations proceed. This simply updates the values of the epistasis coefficients and reference effects to what we would measure in the population if we used the same method of measurement as in the initial population.

Composite Epistasis Parameters

Hansen and Wagner (2001a) and Carter et al. (2005) proposed a set of composite epistasis parameters that were useful in describing the effects of epistasis on the response to selection. The first step in building these parameters is to describe the degree of directional epistasis acting on individual loci. For this purpose, we use a composite measure of average variance-weighted pairwise directional epistasis acting on locus i,

$$ {}^{i}\varepsilon := 2\sum_{j} \frac{{}^{ij}\varepsilon_0 \, {}^{j}V_A}{V_A}, \tag{5} $$

where $2{}^{j}V_A$ is the additive genetic variance contributed by locus j, V_A is the total additive genetic variance, and the ${}^{ij}\varepsilon_0$ are epistasis coefficients measured with reference to a genotype with population mean reference effect at every locus. Throughout this paper, a subscript 0 means that the parameters are measured with reference to this mean genotype. We will also need a measure of how third-order epistasis modifies the interaction between two loci. For this purpose

$$ {}^{ij}\tau := \sum_{k} \frac{{}^{ijk}\varepsilon_0 \, {}^{k}V_A}{V_A} \tag{6} $$

is a composite measure of how locus-directional third-order epistasis modifies the pairwise epistasis between i and j.

These two measures are specific to individual loci or pairs of loci. We also need composite measures of global directional epistasis. For second- and third-order epistasis these are

$$ \varepsilon := 2\sum_{i} \frac{{}^{i}\varepsilon \, {}^{i}V_A}{V_A} = 4 \sum_{i} \sum_{j} \frac{{}^{ij}\varepsilon_0 \, {}^{i}V_A \, {}^{j}V_A}{V_A^2} \quad \text{and} \tag{7a} $$

$$ \tau := 8 \sum_{i} \sum_{j} \sum_{k} \frac{{}^{ijk}\varepsilon_0 \, {}^{i}V_A \, {}^{j}V_A \, {}^{k}V_A}{V_A^3}. \tag{7b} $$

These parameters describe the overall directionality of epistasis and will be small if both positive and negative interactions are common and cancel each other out. We can also define a composite parameter that describes the total strength of epistasis, including nondirectional epistasis,

$$ \theta^2 := 4 \sum_{i} \sum_{j} \frac{{}^{ij}\varepsilon_0^2 \, {}^{i}V_A \, {}^{j}V_A}{V_A^2}. \tag{8} $$

The θ^2-epistasis is closely related to the A × A-epistatic variance as $\theta^2 = 2V_{AA}/V_A^2$. Finally, Carter et al. (2005) found that there is third type of epistasis, intermediate between the directional and nondirectional types, that affects evolutionary dynamics. This is epistasis that is directional on the level of individual loci, but not on the level of the phenotype. This can be measured by the following composite parameter

$$ \omega^2 := 2 \sum_{i} \frac{{}^{i}\varepsilon^2 \, {}^{i}V_A}{V_A} = 8 \sum_{i} \sum_{j} \sum_{k} \frac{{}^{ij}\varepsilon_0 \, {}^{ik}\varepsilon_0 \, {}^{i}V_A \, {}^{j}V_A \, {}^{k}V_A}{V_A^3}. \tag{9} $$

The ω^2-epistasis measures the consistency of the epistatic effects on individual loci. It can be large even in the absence of overall directional epistasis, as long as each locus individually is modified in a consistent direction.

Previous Results on the Response to Standing Variation

As a background to the results on mutation-limited evolution, we briefly review the main analytical results on the response to standing variation with a multilinear architecture from Carter et al. (2005). The single-generation responses to linear directional selection in the mean and additive variance are:

$$ \Delta\bar{z} = \beta V_A + \frac{\varepsilon(\beta V_A)^2}{2} + \frac{\tau(\beta V_A)^3}{6} + o(\beta^3) \quad \text{and} \tag{10a} $$

$$ \Delta V_A = 2\beta\varepsilon V_A^2 + \beta^2(\tau + \omega^2)V_A^3 + 2\beta \sum_{i} {}^{i}C_3 - 2\beta^2 \sum_{i} {}^{i}C_2^2 + 4\beta^2 V_A \sum_{i} {}^{i}\varepsilon \, {}^{i}C_3 + o(\beta^2), \tag{10b} $$

where β is the selection gradient, and ${}^{i}C_2$ and ${}^{i}C_3$ are the second and third cumulant of allelic reference effects at locus i. All parameters are measured with reference to a genotype in which the allelic effects at all loci are set to their population mean.

These equations are derived under the assumption of Hardy-Weinberg and linkage equilibrium, but extensive simulations showed that they predicted the selection response very well even when linkage disequilibrium was allowed to build up (Carter et al. 2005). In fact, even the reduced equations

$$ \Delta\bar{z} = \beta V_A \tag{11a} $$

$$ \Delta V_A = 2\beta\varepsilon V_A^2 + 2\beta \sum_{i} {}^{i}C_3, \tag{11b} $$

yield a good quantitative prediction of the response. The importance of the third cumulant also appears to be an indirect consequence of directional epistasis, as stronger skew evolves with directional epistasis.

What is particularly noteworthy with these equations is that the θ^2-parameter does not appear. More generally, any epistatic term for any moment in the selection-response equations reflects directional epistasis (for formalization and proof of this claim see Carter et al. 2005, appendix B). Thus, we reach a robust theoretical prediction that only directional forms of epistasis affects the response of additive genetic variance to linear selection.

Simulation Methods

Individual-based computer simulations were used to complement the analytical arguments below. The results we present are averages over 15 replicate runs with the same parameter values. For each replicate run, we started by speci-

fying the epistasis coefficients (second and/or third order). Unless otherwise stated, we drew these from a normal distribution with specified mean and variance. We then specify the population by initializing N diploid individuals with n loci divided between two identical genders. All alleles are identical in the starting population.

To form a new generation we first chose N mating pairs with replacement by sampling one individual of each gender with probabilities proportional to their relative fitness in the population. The fitness function we used to specify the fitness of an individual with phenotype z was $W(z) = e^{\beta z}$, where β is a selection parameter, which we set to 0.1. This fitness function approximates linear selection on individual mutations and keeps the selection gradient constant as the mean phenotype changes. For each mating pair, we formed a single progeny by drawing one allele at random from each parent for each locus. We thus assume free recombination.

Mutation was allowed to happen with a specified probability at each allele in the new offspring. When a mutation appeared, the mutated allele was given a reference effect equal to the value of its parent allele plus a normally distributed random variable with mean zero and a specified variance, which we kept constant across loci. We always used the same mutation probability (0.001) for all loci. This mutation rate is several orders of magnitude higher than what is realistic. This was done to speed up simulations and is unlikely to affect our qualitative results. Note, however, that the characteristic time scale of the phenomena that appear in our simulations should therefore not be taken as predictive. Note also that this time scale is set by the product of mutation rate and population size, and that population sizes are small in our simulations (usually $N = 1000$). In general, we have found our results to be qualitatively similar for different choices of population sizes and numbers of loci, and we therefore do not emphasize these parameters in the results we present here.

Measuring Epistasis in the Simulations

Pairwise epistasis coefficients have units equal to the inverse of the trait units, while an r-order epistasis coefficient has units equal to the inverse of the trait units to the power of $r - 1$. We need to take this scale dependency into account when comparing epistasis coefficients over time. We do this by considering the effect of the epistasis coefficient when combined with an average mutation. The epistasis factor describing the effect of a mutation at locus i with reference effect ${}^{i}m$ on subsequent mutations at locus j is

$$ {}^{i\to j}f = 1 + {}^{ij}\varepsilon\, {}^{i}m. \tag{12}$$

Thus, if the mutation effect is 0.1 (on some scale), an epistasis coefficient of 1.0 (on the corresponding scale) means that the epistasis factor for this interaction is 1.1, and thus that the average mutation at locus i will increase the effect of a subsequent mutation on locus j with 10%. To monitor the strength of epistasis over time, we will plot the average pairwise epistasis factor

$$ f = 1 + \frac{1}{2}\langle {}^{ij}\varepsilon_0({}^{i}\bar{m}_0 + {}^{j}\bar{m}_0)\rangle, \tag{13}$$

where the expectation is taken over all pairwise interactions, and ${}^{i}\bar{m}_0 = \sqrt{2\,{}^{i}\sigma^2_{m0}/\pi}$ is the average (absolute) effect of a mutation at locus i, if the effect is drawn from a normal distribution with mean zero and variance ${}^{i}\sigma^2_{m0}$. Recall that the subscript 0 signifies that these parameters are measured with reference to the mean genotype. The f is a scale-free measure of the directional effects of average mutations on other mutations.

Results

Evolution of the Trait Mean

In Figure 1 we illustrate the dynamics of the trait mean over 15,000 generations with different genetic architectures in a population of 1000 individuals. This extends the basic results of Carter et al. (2005) for pairwise epistasis to mutation-limited evolution. Initially, positive directional epistasis leads to an accelerated response, whereas negative directional epistasis leads to canalization and a near standstill of evolution. Carter et al. (2005) found that nondirectional epistatic architectures behaved almost exactly like additive architectures. On the much longer time scales studied here, nondirectional epistasis also eventually generates elevated responses. As discussed in more detail below, this indicates that nondirectional architectures are not completely stable. Furthermore, we find that the initial canalization generated by negative epistasis will eventually be broken, and increased evolvability will evolve. This is clearly seen for strong negative epistasis in Figure 1B, but it also will eventually happen with weaker negative epistasis (see below).

Initially, third-order epistasis has very small effects on the response, but after a few thousand generations (with the parameter values in Fig. 1) it starts to become important, and by 15,000 generations it profoundly affects the dynamics. Again, positive directionality elevates the response, and negative directionality leads to canalization.

Evolution of Mutational Effects

The effect of a given mutation in a background g is ${}^{g\to i}f\,{}^{i}m$, where ${}^{i}m$ is the effect of the mutation in the reference background. If we let ${}^{i}m_0$ be the effect of an (arbitrary) mutation at locus i, as measured with reference to the population mean genotype, then the change from generation to generation in this effect is (Appendix 1):

$$ \Delta {}^{i}m_0 = {}^{\bar{g}'\to i}f\,{}^{i}m_0 - {}^{i}m_0 \approx {}^{i}m_0\,{}^{i}\varepsilon\beta V_A, \tag{14}$$

where ${}^{\bar{g}'\to i}f$ is the epistasis factor showing the effect of the mean genotype after selection, $\bar{g}'$, on locus i. Thus, mutational effects will evolve whenever there is directional epistasis acting on the locus (as measured by ${}^{i}\varepsilon$). If selection is weak, the rate of evolution of mutational effects is proportional to the selection gradient and the additive genetic variance in the population. The evolution of mutational effects over 15,000 generations is illustrated in Figure 2. Note that positive directional epistasis will increase the effects of mutations and that negative directional epistasis will decrease the effects of mutations. Directional higher-order epistatic effects work in the same way, but are proportional to higher

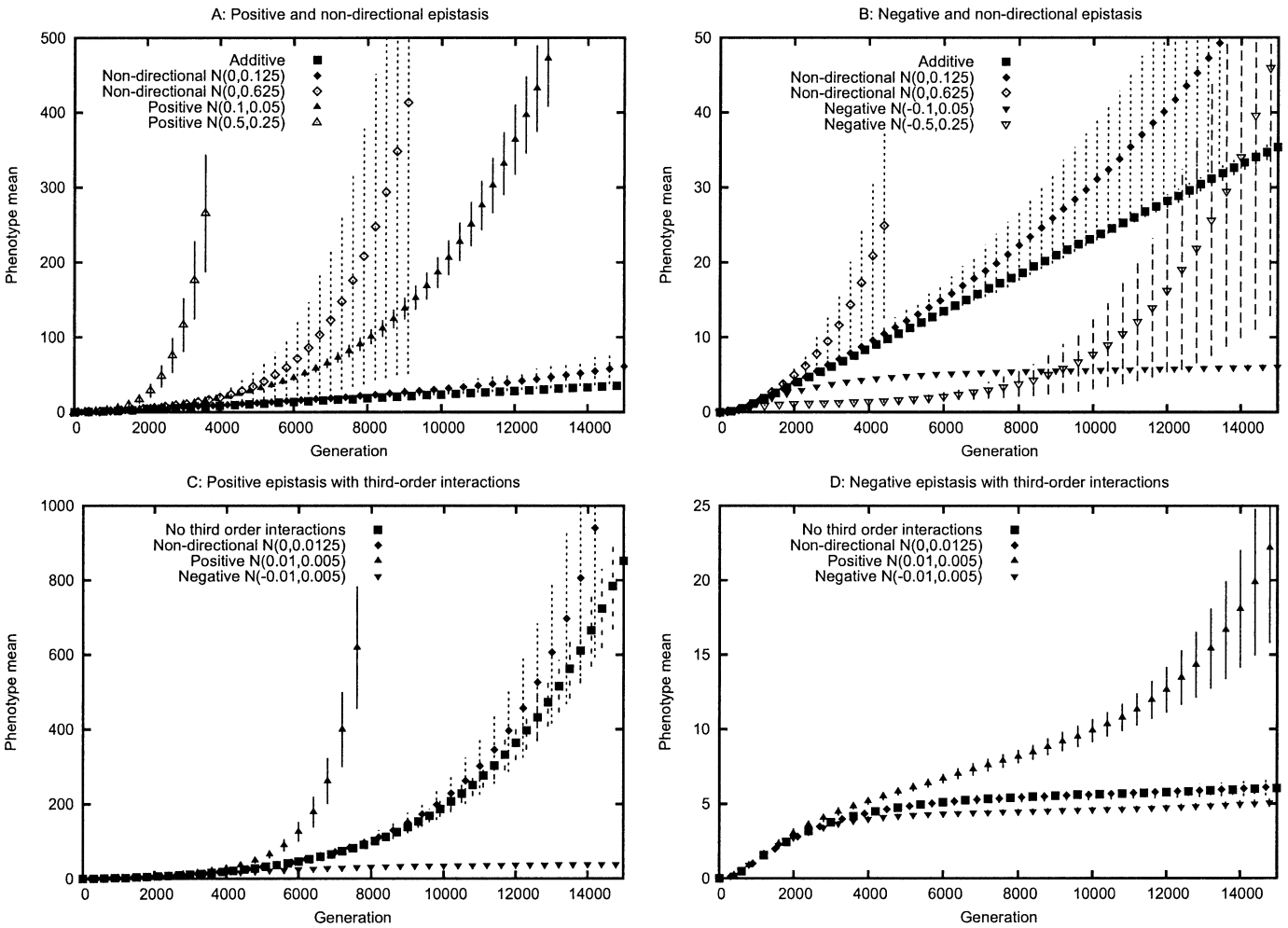

FIG. 1. Evolution of the trait mean. Averages and standard deviations (vertical bars) of 15 sample runs over 15,000 generations of different pairwise epistatic architectures are shown in panels (A) and (B), based on populations of 500 males and 500 females under exponential selection with $\beta = 0.1$. There are 20 loci, and the pairwise epistasis coefficients are drawn from a normal distribution with mean and standard deviation as specified in the figure: $N[(\bar{\varepsilon}, \mathrm{SD}(\varepsilon))]$. Mutation rates are 0.001 per allele per generation, and mutations are drawn from a normal distribution with mean zero and standard deviation 0.025. (A) Positive pairwise epistasis of two strengths with nondirectional epistasis and additive model. (B) Negative pairwise epistasis of two strengths with nondirectional epistasis and additive model. The strengths of nondirectional epistasis are chosen so that the average absolute values of the epistasis coefficients are the same as in the directional cases. (C) Positive pairwise epistasis ($\bar{\varepsilon} = 0.1$, $\mathrm{SD}(\varepsilon) = 0.05$) with three types of third-order epistasis. (D) Negative pairwise epistasis ($\bar{\varepsilon} = -0.1$, $\mathrm{SD}(\varepsilon) = 0.05$) with three types of third-order epistasis.

powers of the selection gradient (Appendix 1), and will be less important under weak selection.

The Evolution of Pairwise Epistasis

We have seen that the evolution of mutational effects depends on the directionality of epistasis acting on the locus, but to understand the long-term dynamics we must ask how the epistasis itself evolves. On a superficial level the composite epistasis coefficients, such as ${}^{i}\varepsilon$, change due to changes in allelic variances, because they are variance-weighted averages of the individual epistasis coefficients. On long time scales, however, these temporary changes are less important than changes in the epistasis coefficients themselves, which are generated by the evolving reference genotype. We now proceed to study these changes in more detail, and the basic dynamics are illustrated in Figure 3, where we plot the average pairwise epistasis factors, which may be thought of as scale-independent epistasis coefficients. The dynamics of unscaled epistasis coefficients are qualitatively similar.

In Appendix 1 we show that the change in a pairwise epistasis coefficient over an episode of selection is

$$\Delta^{ij}\varepsilon_0 = {}^{ij}\varepsilon_0\left(\frac{\bar{g}'\to{}^{ij}f - \bar{g}'\to{}^{i}f\ \bar{g}'\to{}^{j}f}{\bar{g}'\to{}^{i}f\ \bar{g}'\to{}^{j}f}\right)$$
$$\approx \beta V_{\mathrm{A}}[{}^{ij}\tau - {}^{ij}\varepsilon_0({}^{i}\varepsilon + {}^{j}\varepsilon)], \qquad (15)$$

where ${}^{ij}\tau$ (eq. 6) is a composite measure of locus-directional third-order epistasis acting to modify the pairwise epistasis between i and j. The effect of third-order epistasis is thus straightforward: Positive third-order epistasis makes positive changes in pairwise epistasis coefficients, whereas negative third-order epistasis makes negative changes in pairwise epistasis coefficients. To understand the second term in equation

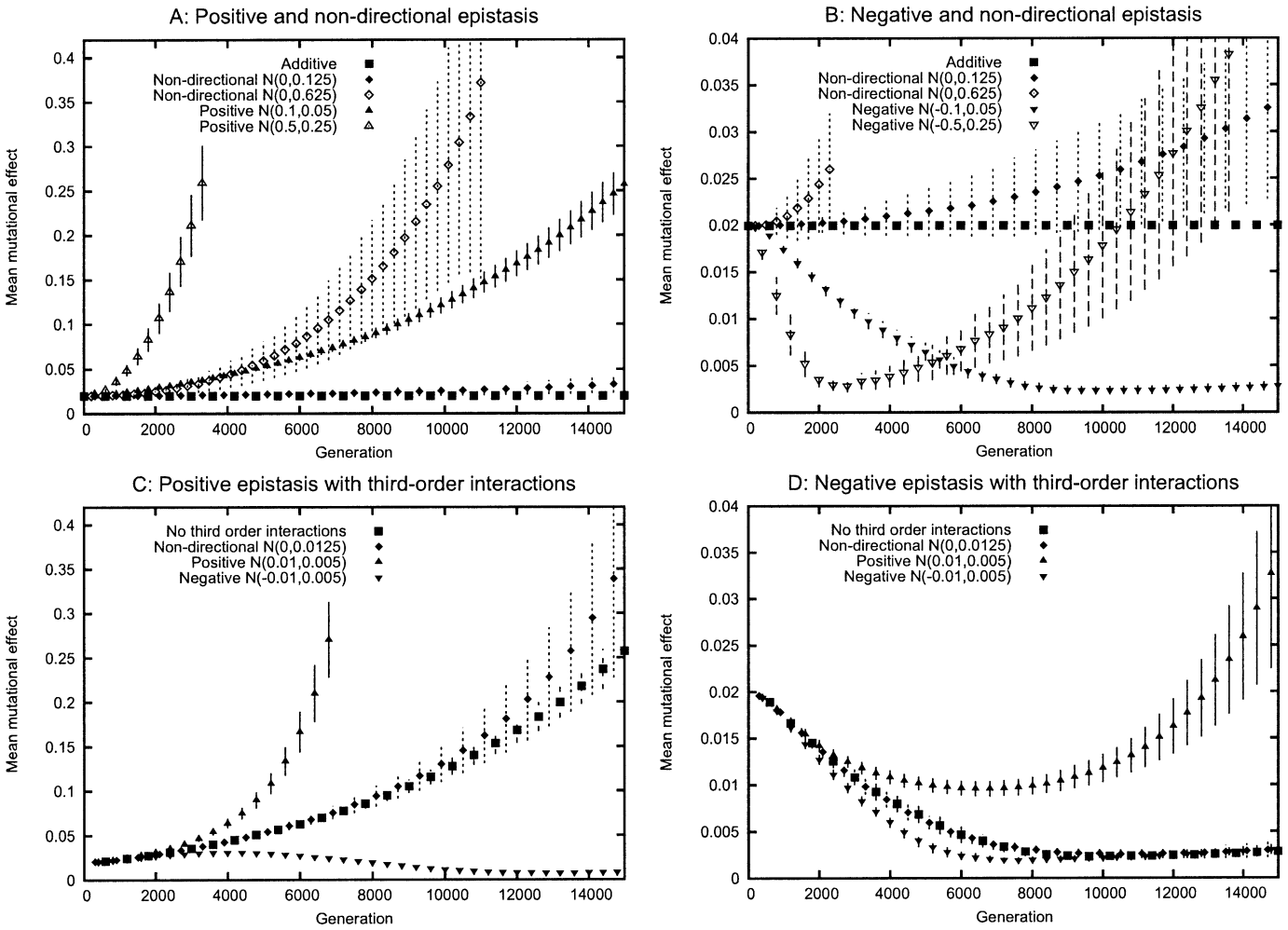

FIG. 2. The evolution of the average absolute value of a new mutation over 15,000 generations of evolution. (A) Positive and nondirectional pairwise epistasis, each of two strengths. (B) Negative pairwise epistasis of two strengths together with (the same) nondirectional epistasis. (C) Positive pairwise epistasis with positive, negative, and nondirectional third-order epistasis. (D) Negative pairwise epistasis with positive, negative, and nondirectional third-order epistasis. Parameter values and symbols are as in Figure 1.

(15), we need to remember that we are looking at epistasis measured with reference to the population mean genotype, and that pairwise epistasis coefficients may change because the reference is changing. The sign of this change is determined by the sign of the parameter $-{}^{ij}\varepsilon_0({}^{i}\varepsilon + {}^{j}\varepsilon)$. This shows that if the sign of the epistasis coefficient agrees with the sign of the sum of the locus-directional epistasis acting on the two loci, then the epistasis coefficient will decrease; that is, positive epistasis will become weaker and negative epistasis will become stronger if the signs agree.

In general, we expect the signs of ${}^{ij}\varepsilon_0$ and ${}^{i}\varepsilon + {}^{j}\varepsilon$ to agree when there is a pattern of directional epistasis. If positive epistasis predominates, then for most combinations of loci both ${}^{ij}\varepsilon_0$ and ${}^{i}\varepsilon + {}^{j}\varepsilon$ will be positive; if negative epistasis predominates, then they both will tend to be negative. This predicts that positive directional epistasis will weaken in the absence of higher-order epistasis (as seen in Fig. 3A), while negative directional epistasis will become stronger (as seen in Fig. 3B). This effect will be counteracted by positive third-order epistasis, and reinforced by negative third-order epistasis (i.e., by the ${}^{ij}\tau$ term; as seen in Fig. 3C, D).

While pure positive epistasis simply evolves toward zero, the evolution of negative epistasis is more complicated. As long as the epistasis is relatively weak and no loci are strongly canalized, the simulations follow the predictions from the theory in that negative epistasis tends to get stronger in the absence of higher-order epistasis, with this pattern being reinforced by negative third-order epistasis and counteracted by positive third-order epistasis (see Fig. 3D). But as negative epistasis coefficients become stronger, they start to behave erratically with large jumps in value, which are apparent even in averages over many runs (Fig. 3). This is caused by some epistasis factors being in the vicinity of zero. As is clear from the change-of-reference equations, this can generate very large epistasis coefficients and also change their sign.

The evolution of nondirectional epistasis also starts out in accordance with the theory. In the absence of higher-order epistasis there is little systematic change in the epistasis co-

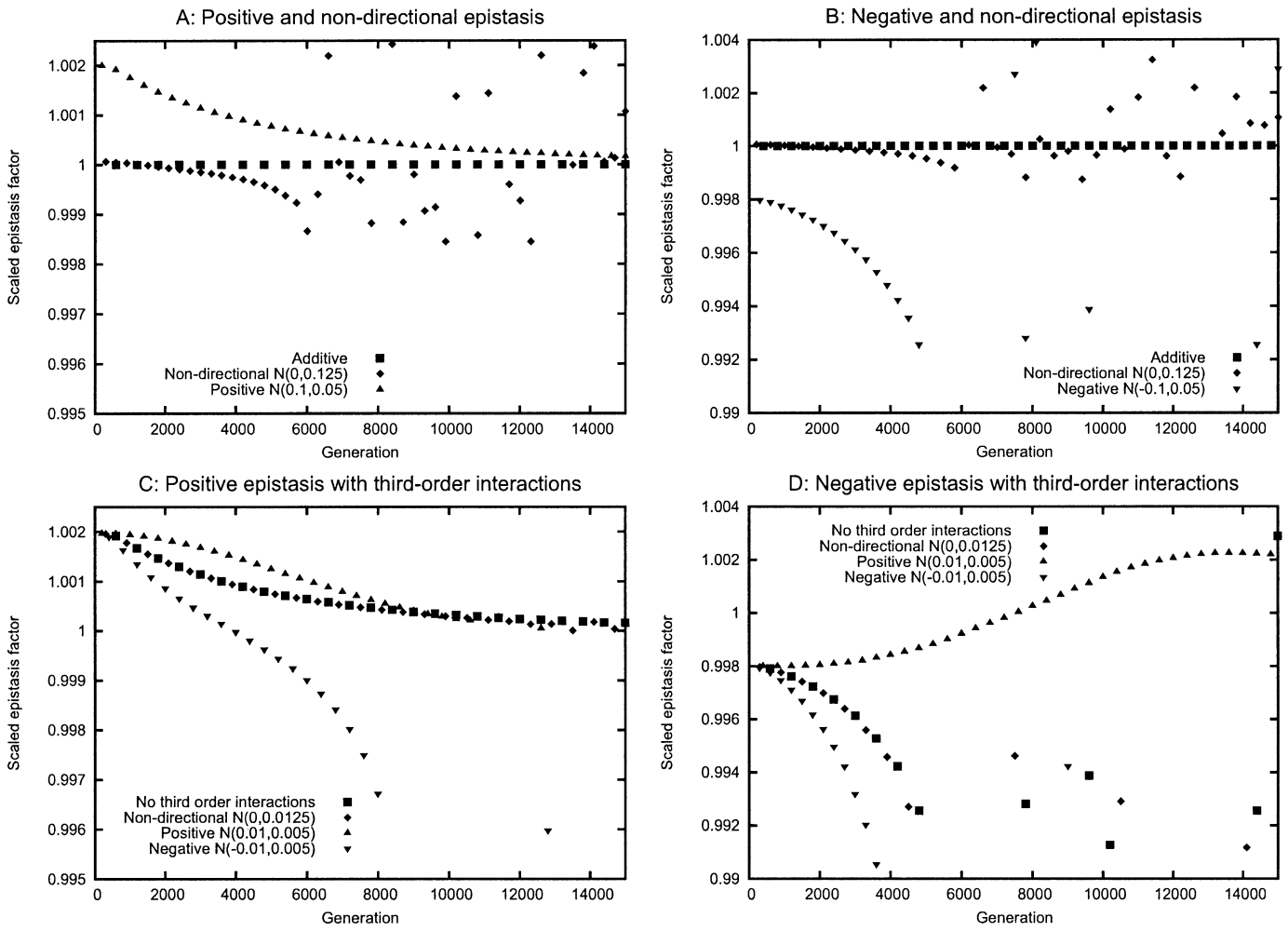

FIG. 3. Evolution of the strength of epistasis. The evolution of the mean pairwise epistasis factor ($f = 1 + \langle {}^{ij}\varepsilon_0({}^{i}\bar{m}_0 + {}^{j}\bar{m}_0)/2\rangle$) over 15,000 generations. (A) Positive epistasis and nondirectional epistasis. (B) Negative and nondirectional epistasis. Note that as negative epistasis gets stronger, it will suddenly reach a point where some loci get completely canalized with very strong epistasis. These will then dominate the average epistasis factor, which starts to fluctuate erratically. The strong epistasis cases are not shown, as they are more extreme cases of the weak epistasis shown. With strong negative or nondirectional epistasis the erratic fluctuations starts very soon (~1000 generations). (C) Positive pairwise epistasis with three types of third-order epistasis. (D) Negative pairwise epistasis with three types of third-order epistasis. Parameter values and symbols as in Figure 1.

efficients, but after a long time, negative epistasis starts to evolve. We think this is due to the presence of some weak directional epistasis caused by mutational stochasticity, genetic drift, or sampling effects in the simulations. In any case, this shows that nondirectional epistatic architectures are not stable. If directional third-order epistasis is present, directional second-order epistasis rapidly appears in accordance with the sign of the third-order epistasis (not shown).

Comparisons of simulations with and without variation in epistasis coefficients are shown in Figure 4. While the evolution of positive epistatic architectures is less affected by variance in epistasis, there are strong effects on the evolution of negative and nondirectional architectures, which respond much more slowly without variance in epistasis. A nondirectional architecture without variance in epistasis is implemented by alternating positive and negative epistasis coefficients of exactly the same magnitude. Note, in particular, the evolution of the weak epistasis cases in Figure 4D. After 100,000 generations, nonvariable nondirectional epistasis still behaves almost exactly like the additive model, and nonvariable negative epistasis remains in an almost completely canalized state for almost as long. Even these architectures, however, may eventually achieve accelerated evolution (see below).

Evolution of Third- and Higher-Order Epistasis

For the same reason as second-order epistasis evolves in the absence of directional third-order epistasis, the third-order epistasis coefficients also change over an episode of selection according to

$$\Delta^{ijk}\varepsilon_0 = {}^{ijk}\varepsilon_0\left(\frac{\bar{g}'\to ijk f - \bar{g}'\to i f\,\bar{g}'\to j f\,\bar{g}'\to k f}{\bar{g}'\to i f\,\bar{g}'\to j f\,\bar{g}'\to k f}\right)$$
$$\approx -\beta V_A\,{}^{ijk}\varepsilon_0({}^{i}\varepsilon + {}^{j}\varepsilon + {}^{k}\varepsilon), \qquad (16)$$

where $\bar{g}'\to ijk f = 1$ by assumption of no fourth- or higher-order

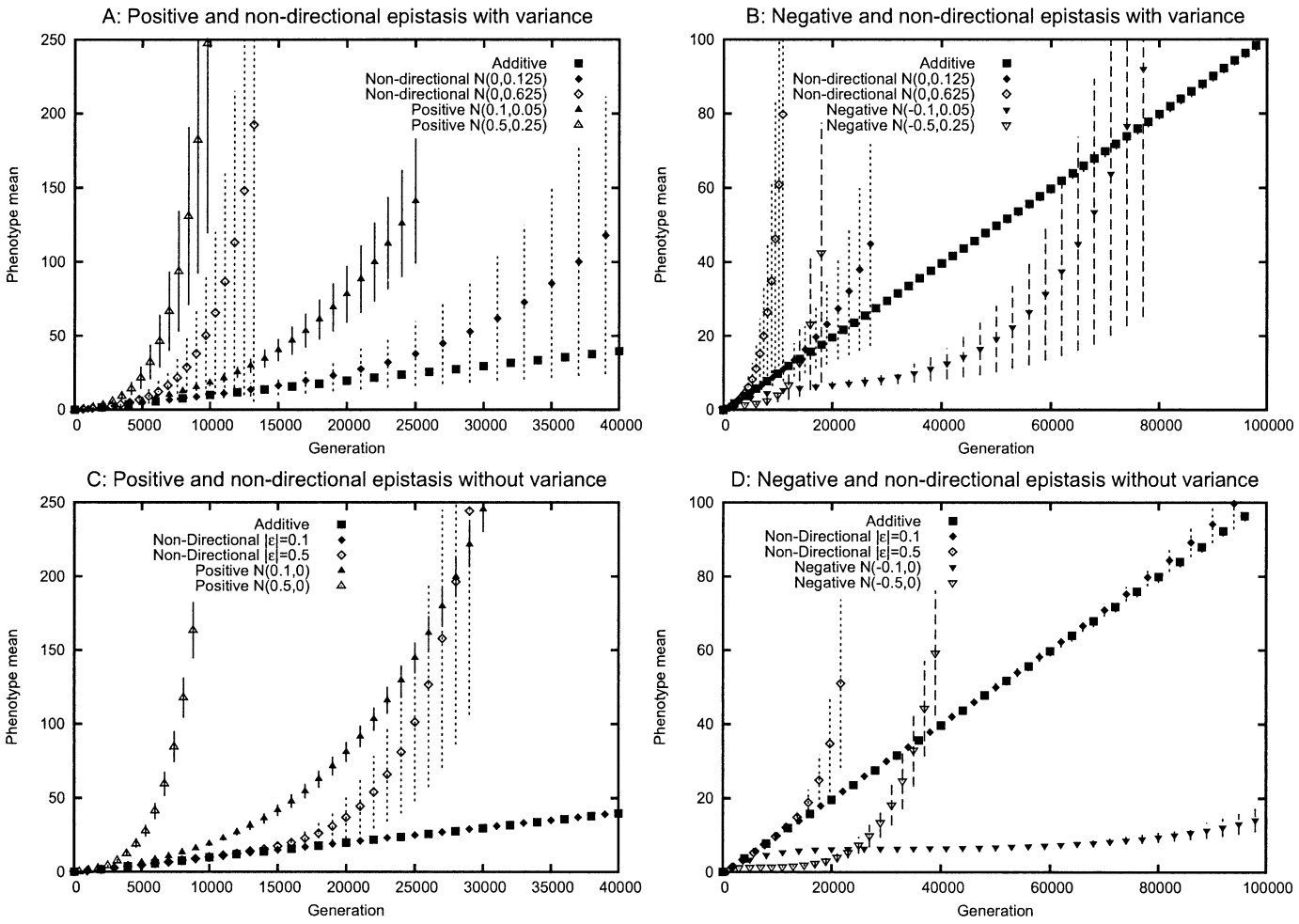

FIG. 4. Comparison of cases with variance in the epistasis coefficients (A, B) with cases in which there is no variance in the epistasis coefficients (C, D). (A) and (C) Positive epistasis of two strengths and nondirectional epistasis. (B) and (D) Negative epistasis of two strengths and nondirectional epistasis. Nondirectional epistasis with no variance is implemented by letting all epistasis coefficients be either plus or minus the same value such that each row in the epistasis matrix adds to zero. Parameters and symbols are as in Figure 1, except that we use five loci and population size is $N = 4000$.

epistasis. By similar arguments to those given above, we can predict that positive directional third-order epistasis tends to decline, while negative directional third-order epistasis tends to be reinforced. The simulations confirm these predictions, but again, we see erratic behavior when loci become canalized by negative epistasis (not shown).

These patterns generalize to higher orders of epistasis (Appendix 1). Positive directional epistasis of any order has an inherent tendency to decrease, whereas negative directional epistasis tends to become stronger (i.e., become more negative). Positive directional epistasis of order r will tend to increase epistasis of order $r - 1$, and negative directional epistasis of order r will decrease it.

Quasi-Equilibrium Genetic Architectures

The above results suggest that directional epistasis cannot be at equilibrium during directional selection. If directional epistasis is present, both mutational effects and epistasis coefficients will change. This leads to the question of whether any genetic architecture with epistasis can be qualitatively invariant under selection. We hypothesize three general types of quasi-equilibria: (1) nondirectional epistatic equilibria; (2) near-additive equilibria in which epistasis is extremely weak relative to mutational effects; and (3) canalized equilibria in which marginal mutational effects disappear and epistasis becomes extremely strong. We now discuss these in turn, and provide some heuristic arguments for their robustness and stability properties.

Clearly, mutational effects will evolve as long as there is locus-directional epistasis (${}^{i}\varepsilon \neq 0$ for some i). The criterion for pairwise epistasis coefficients to be at equilibrium is that ${}^{ij}\varepsilon_0({}^{i}\varepsilon + {}^{j}\varepsilon) = {}^{ij}\tau$. In the absence of directional third-order epistasis (i.e., ${}^{ij}\tau = 0$), this means that nonzero epistasis between locus i and j can be at equilibrium only if locus-directional epistasis acting on the loci exactly cancels (i.e., ${}^{i}\varepsilon = {}^{j}\varepsilon$). Thus, pure nondirectional epistasis will be in equilibrium. There is also a possibility that some alternating locus-directional epistasis may exist in a such equilibrium, but if there is positive directional epistasis on one locus, this locus can only interact with other loci on which there is exactly

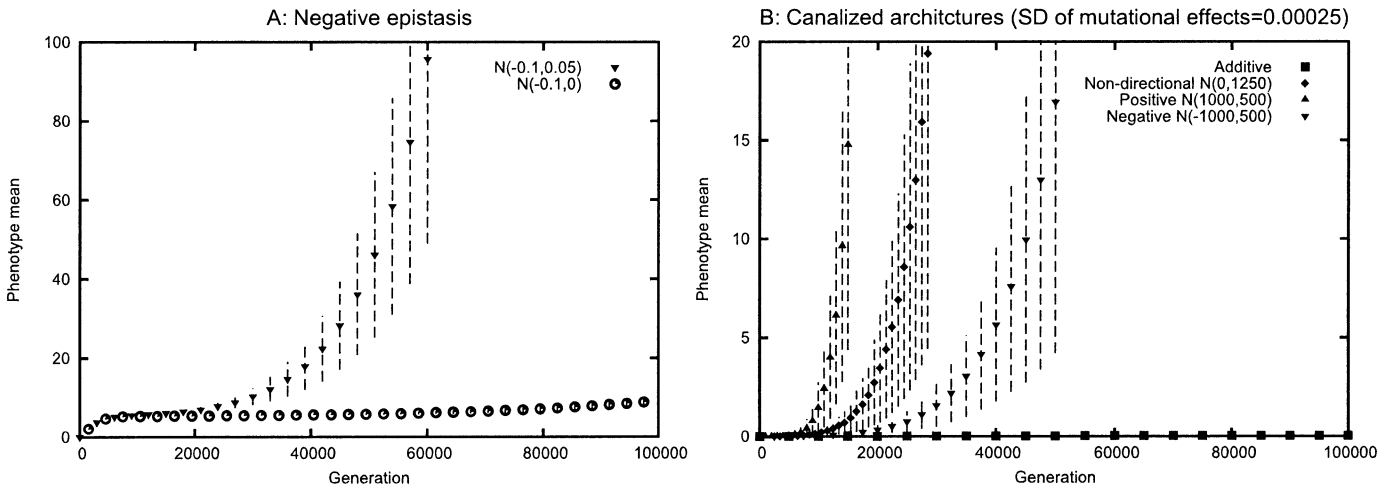

FIG. 5. Canalization and its breakdown. Here we show long-term evolution of the phenotypic mean under (A) negative epistasis, and (B) strong initial canalization. The simulations in (A) show weak negative epistasis with and without variation in epistasis. Note how an initial response gives way to a state of near-complete canalization, which is then eventually broken, so that a new and more rapid response can take place. Without variation in epistasis, the canalization extends for much longer. In (B) we start with very small mutational effects, but proportionally larger epistasis coefficients, so that the epistasis terms are similar to the weak-epistasis cases in the previous simulations. Note how the canalization is broken relatively suddenly and gives way to a rapid response. Parameters and symbols as in Figure 1.

counterbalancing negative directional epistasis; this rules out overall directional epistasis, which is an average over all the ${}^{i}\varepsilon$. If locus-directional third-order epistasis is present (${}^{ij}\tau \neq 0$), directional pairwise epistasis can be at equilibrium, but in the absence of even higher-order epistasis, the directional third-order epistasis itself can only be at equilibrium if there is no directional second-order epistasis. For ${}^{ijk}\varepsilon_0 \neq 0$ equilibria to exist, we must have that ${}^{i}\varepsilon + {}^{j}\varepsilon + {}^{k}\varepsilon = 0$, which again implies that directional epistasis will have to cancel.

Even these nondirectional epistatic equilibria are not generally stable. Because they depend on exact values of composite parameters such as the ${}^{i}\varepsilon$, these equilibria will be perturbed by changes in allelic variances, which will happen in any finite population. The equilibrium set of nondirectional epistatic equilibria will be stable against perturbations that generate positive directional epistasis, but it may be unstable against perturbations that generate negative directional epistasis, since the latter tends to get reinforced. Consistent with this, our simulations show that after some time, negative directional epistasis begins to appear from nondirectional architectures. This average effect, however, is associated with the evolution of very large variation and sign changes in the epistasis coefficients. Some loci therefore also get decanalized, and because mutational effects have a lower bound at zero but no upper bound, the average mutational effect starts to increase. With strong nondirectional epistasis this can happen rather quickly, but weaker and less variable nondirectional epistasis can remain in a quasi-equilibrium for a very long time (as illustrated in Fig. 4D).

Near-additive architectures are stable against positive directional epistasis, which will decrease, but not against negative directional epistasis. This explains the simulations in which we start with positive directional epistasis. In this case, mutational effects increase and epistasis coefficients decrease until we reach a near-additive genetic architecture.

A third possible equilibrium occurs when mutational effects (i.e., ${}^{i}m_0$) evolve toward zero. If all members of an interacting set of loci are canalized, the composite epistasis parameters for these loci, ${}^{i}\varepsilon$, and ${}^{ij}\tau$, go to zero, as allelic variances disappear. Note also that if all loci are canalized we get an equilibrium because $V_A = 0$. To get some insight into the stability of a canalized genetic architecture, we can consider a locus, i, where mutational effects are very close to zero. Then, as shown above (eq. 14), the change in mutational effects is $\Delta {}^{i}m_0 \approx {}^{i}m_0 {}^{i}\varepsilon \beta V_A$. Thus, a negative ${}^{i}\varepsilon$ will decrease the effect and maintain canalization. Negative directional epistasis, however, also will become stronger, and if it gets too strong, the mutational effects may overshoot zero and eventually make canalization unstable. In effect, what happens is that all alleles on the locus change signs in their effect on the trait, thus reversing selection on that locus. This is an example of ''sign epistasis'' (Weinreich et al. 2005). With extremely strong epistasis, the epistasis factors, ${}^{\bar{g}'\to i}f$, may also become negative, which changes the sign of ${}^{ij}\varepsilon_0$ from one generation to the next. This generates some positive epistasis that may fuel further changes. Note that this instability is a result of the sign epistasis implied by multilinearity and that canalization under negative epistasis would be stable if the signs of mutational effects were not allowed to change.

Consistent with the above scenario, our simulations show that initial negative directional epistasis first leads to relatively stable canalization, but after a long time very strong and erratic epistasis appears to interrupt the standstill and generate renewed change. This is illustrated in Figures 5 and 6. Notice in particular what happens, in Figures 6B and 6D, when we start with canalized architectures in which all mutational effects are very small, but the epistasis coefficients are correspondingly larger to keep epistasis terms similar to the weak epistasis simulations in Figures 1–4. The canalization is initially stable for a long time, but it eventually breaks down and an accelerating response ensues. This hap-

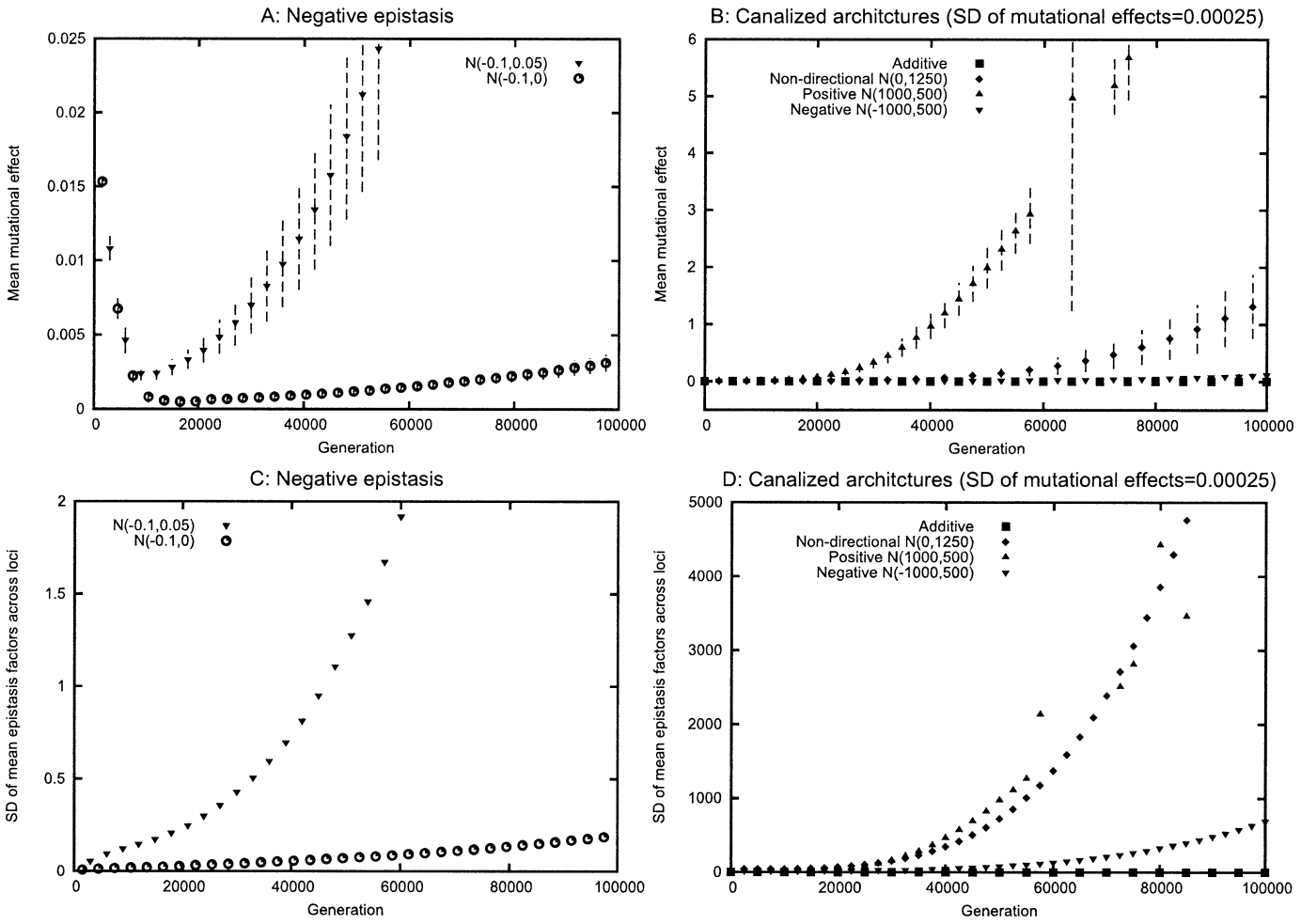

FIG. 6. Evolution of genetic architecture under canalization. Here we show what happens to the genetic architecture in the simulations shown in Figure 5. (A) and (C) correspond to the negative epistasis in Figure 5A and (B) and (C) to the initial canalization in Figure 5B. (A) and (B) show mutational effects. (C) and (D) show the standard deviation of scaled epistasis factors across loci. The averages of the scaled epistasis factors are not shown, as they most of the time remain very close to one with some very large occasional deviations in all these simulations. Thus, enormous nondirectional epistasis evolves in the canalized architectures. Note also how the mutational effects remain canalized with initial negative epistasis, and that the response seen for this case in Figure 5B must therefore be due solely to epistasis. Parameters and symbols as in Figure 1.

pens more quickly if positive epistasis was initially present. These responses, however, are driven more by the evolution of extremely strong and largely nondirectional epistasis than they are by decanalization of mutational effects. Note, in particular, how mutational effects barely increase in simulations that started with negative epistasis.

Long-Term Constraints

The above analysis is based on using the mean genotype in the population as reference genotype. This has the advantage of describing the genetic architecture and changes in genetic architecture in terms of parameters that are operational in the sense that they, in principle, can be measured in the evolving population. We can of course also describe the process in terms of the original parameters set for the initial population. These parameters would be measurable, in principle, if the original population was available for genetic analysis (i.e., if genotypes from the evolving population could be substituted into the ancestral population).

From the vantage point of the initial population, all the epistasis coefficients of all orders remain as constant parameters. If there are no restrictions on the allelic reference effects, as in our simulations, this means that the dynamics eventually will become dominated by the epistasis terms of highest order. This illuminates the long-term potential of different initial genetic architectures. Starting with pure positive directional epistasis (on the highest order) there are no constraints on evolution of the character, as all highest-order terms can have the same sign. This is not the case with nondirectional or negative initial epistasis. Consider an epistasis term, like ${}^{ij}\varepsilon\, {}^{i}y\, {}^{j}y$, where there is negative epistasis (${}^{ij}\varepsilon < 0$), then ${}^{i}y$ and ${}^{j}y$ need have opposite signs to make the term contribute to positive character change. This induces constraints on the contributions of these loci to other epistasis terms, such that not all terms can contribute to character evolution. However, it is always possible to find some combination of epistasis terms that contribute positively to the trait. Thus, a completely canalized architecture cannot be globally stable under multilinear epistasis.

In Appendix 2 we show, in the case of pairwise epistasis, that the asymptotic response of the trait mean is an exponential, where the exponent is proportional to the largest eigenvalue of the epistasis matrix (the matrix with ${}^{ij}\varepsilon$ as its ijth element). Because this eigenvalue must be positive, no absolute constraints can exist. The largest eigenvalue, however, is strongly affected by the pattern of epistasis and will usually be much larger with positive than with negative initial epistasis. As a basic comparison of positive and negative epistasis: If all ${}^{ij}\varepsilon = \varepsilon > 0$, then the largest eigenvalue is $\lambda_{max} = (n - 1)\varepsilon$, where n is the number of loci, whereas if all ${}^{ij}\varepsilon = -\varepsilon < 0$, then $\lambda_{max} = \varepsilon$. Thus, although an accelerating response is possible with all types of multilinear epistasis, negative epistasis is severely constraining relative to positive epistasis.

Discussion

Dating back to the work of Waddington (1942, 1957), it has become more or less accepted that stabilizing selection should lead to canalization, while directional selection should lead to decanalization. Recent theoretical results challenge both of these ideas. The notion that stabilizing selection is canalizing has been supported by the general empirical observation that wild types tend to be less phenotypically variable than mutants (for reviews, see Schaarloo 1991; Moreno 1994; Gibson and Wagner 2000; Dworkin 2005; Flatt 2005; Wagner 2005; Hansen 2006), but it has been shown that a release of genetic variation does not imply that the wild type is mutationally canalized (Hansen and Wagner 2001a; Bergman and Siegal 2003; Hermisson and Wagner 2004). The prediction that stabilizing selection should favor canalization has some theoretical support (Layzer 1980; Cheverud 1984; Gavrilets and Hastings 1994; A. Wagner 1996; Wagner et al. 1997; Rice 1998, 2002; Siegal and Bergman 2002; Azevedo et al. 2006), but direct selection for canalizing modifiers is often weak (e.g., Wagner et al. 1997; Proulx and Phillips 2005). In addition, Hermisson et al. (2003) showed that the multilinear model under stabilizing selection does not generally lead to the most canalized state and could lead to decanalization, because loci typically interfere with the canalization of each other. Decanalizing effects of directional selection have been suggested by several authors (Layzer 1978, 1980; G. P. Wagner 1996; Wagner et al. 1997; Rice 1998). Kawecki (2000), in a simulation study with a modifier model under fluctuating directional selection, found support for decanalization if the period of fluctuation was long. The results presented here, however, as well as those of Hansen and Wagner (2001a) and Carter et al. (2005), showed that even this is not generally the case, because directional selection leads to canalization when negative epistasis is predominant. We have shown that whether we should expect directional selection to be canalizing or decanalizing depends on the prevalence of positive versus negative epistasis.

The most fundamental aspect of our results is that the evolution of allelic and mutational effects primarily depends on the directionality of epistasis. Indeed, it is intuitively obvious that gene effects will increase if genes systematically reinforce each other in the direction of evolution, and that canalization will ensue if they systematically reduce each other's effects. These results are generic descriptors of second-order dynamics in the same sense as the additive model gives a generic first-order description of the response to selection.

The evolution of the epistatic effects is more complicated. We have shown that there is an inherent tendency for positive epistasis to weaken and for negative epistasis to strengthen, relative to additive effects and under directional selection, but this can be modified by directional third-order epistasis. In general, positive directional epistasis of order r will induce positive changes in epistasis of order $r - 1$, while negative directional epistasis of order r will induce negative changes in epistasis of order $r - 1$, but beyond this, there is an inherent tendency for negative changes of epistasis coefficients of all orders. An interesting implication of this result is that the effect of natural selection on genetic architecture is as dependent on the structure of the gene interactions themselves as they are on the nature of the selective force. The genetic architectures resulting from either stabilizing (Hermisson et al. 2003) or directional selection are not predictable without knowing the statistically prevailing form of gene interaction.

The evolution of epistasis in the absence of higher-order epistasis may seem puzzling unless one remember that it is strictly a consequence of the reparameterization of the model relative to an evolving reference population. The tendency to reduce positive epistasis can be understood as a side effect of the decanalization of loci with positive interactions. The resulting increase in their additive effects means that smaller epistasis coefficients are necessary to account for their interaction. Similarly, the canalization of negatively interacting loci means that their negative epistasis coefficients must have larger absolute values to account for their interaction. In the absence of an overall directionality, however, a pair of positively interacting loci are not necessarily decanalized, and their epistasis coefficient may then be as likely to increase as to decrease. We remind the reader that these changes are fully observable in the sense that different epistasis coefficients would be obtained by fitting the model to the population before and after a period of evolution.

Negative epistatic architectures evolve toward a state of canalization and strong epistasis. In the multilinear model, these architectures are not globally stable, however, and a subset of positive epistatic interactions will eventually emerge, as some loci change the sign of their effects on the trait, so that previously favored alleles are now selected against. Weinreich et al. (2005) termed cases in which the genetic background changes the sign of an allele's effect on fitness as ''sign epistasis.'' Naturally, sign epistasis has dramatic effects on the evolutionary trajectory. Note also that variation in epistatic effects is important in generating these revolutions (as suggested by Phillips et al. 2000). However, the sign epistasis and the resulting instability of canalization are perhaps the least realistic general implication of multilinearity. If we imagine that allelic effects could be reduced toward zero, but never change sign, then a negative epistatic architecture could result in stable canalization, and absolute epistatic constraints would appear and be maintained by continued directional selection. For these reasons, we suggest that the tendency for negative epistasis to strengthen means that there is a realistic possibility for directional selection to

result in stable canalization. This may eventually lead to an epistatic constraint on further evolution, and we may hypothesize that there exist traits that have been stopped in their evolutionary tracks by epistatic constraints built by prolonged directional selection. Fitness components suggest themselves as likely candidates (de Visser et al. 2003), although little additive variance would be expected in this situation.

While the evolution of epistasis is a topic practically absent from the classical population-genetics literature, several recent papers have begun to illuminate the issue (for review see Hansen 2006). Liberman and Feldman (2005) studied the evolution of epistasis in a polymorphic two-locus system with a modifier approach. They found that there was selection for the epistatic interaction to strengthen when the two loci were kept in a polymorphic equilibrium by balancing selection. Their results are not directly comparable to ours, as both the pattern of selection and the genetic architecture are different and they measured epistasis with reference to an a priori genotype, but the key to their result appears to be that the mean fitness in their model was an increasing function of their epistasis parameter. Our results, however, show that it is not generally true that the pattern of epistasis that increases population fitness, positive epistasis in our case, will be favored. Azevedo et al. (2006) investigated the evolution of epistasis in a gene-network model developed by A. Wagner (1996). In this model there is stabilizing selection on the pattern of gene expression, but Azevedo et al. focused on the evolution of epistasis for fitness. Consistent with our results they found a tendency for more negative epistasis to appear in the evolving population. They interpreted this as being an indirect consequence of selection for robustness, as negative epistasis is correlated with canalization in their model. Similar results have been reported from the evolution of RNA secondary structure and digital organisms (Wilke and Adami 2001; Misevic et al. 2006). Our results suggest that the evolution of epistasis in the negative direction may be generic and not dependent on the coevolution of robustness. Hermisson et al. (2003) found that directional epistasis tends to disappear under stabilizing selection. They studied the behavior of the multilinear model in a balance between mutation and stabilizing selection. With pairwise epistasis, they found that the genetic architecture evolved toward a state where the directional epistatic effects (i.e., the $^{i}\varepsilon$) on all loci would align and be proportional, but opposite in sign, to the difference between the population mean and the optimum. Due to the concavity of the fitness function, remaining epistasis would then generate negative epistasis for fitness, as found by Azevedo et al. (2006). One interpretation of these results is that we should expect weak directional epistasis in traits that have been kept for a long time in mutation-selection balance. If strong directional epistasis were to be observed in traits under either stabilizing or directional selection, however, it may be taken to reflect an underlying epistatic constraint on adaptation.

We also note that Elena and Lenski (2001) conducted an experiment with *Escherichia coli* that is analogous to our simulations. They compared the fitness effects of specific insertion mutations in a population that had been selected in a defined environment for 10,000 generations with the effects of the same mutations in the ancestral background. Four of 12 mutations showed noticeable epistatic interaction with the background, but because two of these had increased fitness and two reduced fitness in the derived background, there was no indication of directional epistasis. This is consistent with our model in that nondirectional epistasis is not changing under directional selection.

To interpret our results in a broader context, it is essential to realize that the multilinear epistatic model is an approximation to a highly complex genotype-phenotype map. Multilinearity entails that all alleles at a locus are modified by the same epistasis factor. This allows stretching and compression of differences of allelic effects, but not allele-specific interactions across loci. This limits the possibilities for the evolution of coadapted gene complexes, where only specific allele combinations convey high fitness, and it limits dominance to evolve only in proportion to the evolution of additive effects (Hansen and Wagner 2001a). The Gaussian mutation model with its continuum of unrestricted allelic effects is another critical assumption of our setup. If only a finite or restricted set of allelic values are possible (with respect to any given reference genotype), this will constrain the potential for evolution of genetic architecture and eventually result in an absolute genetic constraint, as it does in the additive model (Cockerham and Tachida 1987).

The multilinear model will be a good local approximation to any smooth genotype-phenotype map that can be approximated with a Taylor expansion. Our predictions for the short-term evolution of allelic and epistatic effects will be quite general and robust in the sense that they derive from these local properties. The long-term predictions involving large changes in phenotype, however, are much more restrictive, as they depend on continuous global validity of the multilinear form. Therefore, the global predictions should be viewed as null models. For example, the eventual outcome of directional selection on a positive epistatic architecture is a rapidly evolving near-additive architecture in which epistatic effects are weak relative to additive effects. Reaching this state, however, would involve unrealistically large changes in phenotype. In reality, a variety of constraints could render both the directional selection assumption and the multilinearity assumption dubious on this scale. However, we have shown that a canalized state could be relatively stable in the face of directional selection if it were to be reached. The fact that directional epistasis is generally unstable under directional selection may help explain why it is rarely observed in data. For example, the accelerating response predicted by positive directional epistasis has never been observed in artificial selection experiments (Johnson and Barton 2005).

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Appendix 1: Evolution of Genetic Architecture

If the trait is under linear directional selection with selection gradient β and all alleles are independent of each other, then the change in the mean and variance of reference effect at locus i over one generation are

$$\Delta\langle {}^{i}y\rangle = 2s\,{}^{i}C_2 \quad \text{and} \tag{A1}$$

$$\Delta\,{}^{i}C_2 = s\,{}^{i}C_3 - s^2\,{}^{i}C_2^2, \tag{A2}$$

where $s = \beta\,\langle {}^{g\to i}f\rangle$ is the selection coefficient, $2\,{}^{i}C_2$ is the additive genetic variance in the locus reference effect, and ${}^{i}C_3$ is the third cumulant of the allelic distribution at locus i (Bürger 2000). The change in the mean epistasis factor is best expressed by measuring effects with reference to a genotype where the effect of each locus is at the population mean. Thus, the mean reference effects are all zero, and the average epistasis factors after selection can be expressed as

$$\langle {}^{g\to i}f\rangle' = 1 + \sum_j {}^{ij}\varepsilon_0\Delta\langle {}^{j}y\rangle + \frac{1}{2!}\sum_j\sum_k {}^{ijk}\varepsilon_0\Delta\langle {}^{j}y\rangle\Delta\langle {}^{k}y\rangle + \cdots. \tag{A3}$$

In the mean reference under linkage equilibrium, the mean epistasis factors before selection are unity. Using this and equation (A1), we obtain

$$\begin{aligned}\langle {}^{g\to i}f\rangle' &= 1 + 2\beta\sum_j {}^{ij}\varepsilon_0\ {}^{j}V_{\mathrm{A}} + \frac{1}{2!}(2\beta)^2\sum_j\sum_k {}^{ijr}\varepsilon_0\ {}^{j}V_{\mathrm{A}}\ {}^{k}V_{\mathrm{A}} + \cdots \\ &= 1 + {}^{i}\varepsilon\beta V_{\mathrm{A}} + \frac{{}^{i}\tau\beta^2V_{\mathrm{A}}^2}{2!} + o(\beta^2).\end{aligned} \tag{A4}$$

Higher-order epistasis factors describing the modification of the interaction between a set of loci, J, are defined in Hansen and Wagner (2001a), as

$${}^{g\to J}f = \frac{\sum_{K\in P(g)} {}^{J\cup K}\varepsilon\prod_{j\in K}{}^{j}y}{{}^{J}\varepsilon}, \tag{A5}$$

where $P(g)$ is the power set of g, and the index set $J \cup K$ is the union of indices from J and K. By the same argument as for the first-order epistasis factor, we obtain, to the first order,

$$\langle {}^{g\to J}f\rangle' = \frac{\sum_{K\in P(g)} {}^{J\cup K}\varepsilon_0\prod_{j\in K}\Delta\,{}^{j}y}{{}^{J}\varepsilon_0} \approx \frac{\beta\sum_{k\notin J} {}^{J\cup k}\varepsilon_0\,{}^{k}V_{\mathrm{A}}}{{}^{J}\varepsilon_0} + o(\beta). \tag{A6}$$

For second-order epistasis factors, this gives

$$\langle {}^{g\to ij}f\rangle' = \frac{{}^{ij}\tau\beta V_{\mathrm{A}}}{{}^{ij}\varepsilon_0} + o(\beta), \tag{A7}$$

where ${}^{ij}\tau = \Sigma_k\,{}^{ijk}\varepsilon_0\,{}^{k}V_{\mathrm{A}}/V_{\mathrm{A}}$.

The changes in epistasis coefficients over an episode of selection are

$$\Delta\,{}^{ij}\varepsilon = {}^{ij}\varepsilon\left(\frac{\bar{g}'\to{}^{ij}f - \bar{g}'\to{}^{i}f\,\bar{g}'\to{}^{j}f}{\bar{g}'\to{}^{i}f\,\bar{g}'\to{}^{j}f}\right), \tag{A8}$$

$$\Delta\,{}^{ijk}\varepsilon = {}^{ijk}\varepsilon\left(\frac{\bar{g}'\to{}^{ijk}f - \bar{g}'\to{}^{i}f\,\bar{g}'\to{}^{j}f\,\bar{g}'\to{}^{k}f}{\bar{g}'\to{}^{i}f\,\bar{g}\to{}^{j}f\,\bar{g}\to{}^{k}f}\right) \quad \text{and} \tag{A9}$$

$$\Delta\,{}^{J}\varepsilon = {}^{J}\varepsilon\left(\frac{\bar{g}'\to{}^{J}f - \prod_{i\in J}\bar{g}'\to{}^{i}f}{\prod_{i\in J}\bar{g}'\to{}^{i}f}\right), \tag{A10}$$

where $\bar{g}'$ represents the new reference genotype in the next generation equal to the (hypothetical) genotype with mean reference effect at all loci. Note that under the assumption of linkage equilibrium ${}^{\bar{g}\to i}f = \langle {}^{g\to i}f\rangle$, and more generally, ${}^{\bar{g}\to J}f = \langle {}^{g\to J}f\rangle$. Thus, we can use equations (A4), (A6), and (A7) in (A8), (A9), and (A10) to obtain

$$\Delta\,{}^{ij}\varepsilon = \beta V_{\mathrm{A}}\left[\frac{{}^{ij}\tau - {}^{ij}\varepsilon({}^{i}\varepsilon + {}^{j}\varepsilon)}{1 + \beta V_{\mathrm{A}}({}^{i}\varepsilon + {}^{j}\varepsilon)}\right] + o(\beta), \tag{A11}$$

$$\Delta\,{}^{ijk}\varepsilon = \beta V_{\mathrm{A}}\left[\frac{{}^{ijk}\tau_4 - {}^{ijk}\varepsilon({}^{i}\varepsilon + {}^{j}\varepsilon + {}^{k}\varepsilon)}{1 + \beta V_{\mathrm{A}}({}^{i}\varepsilon + {}^{j}\varepsilon + {}^{k}\varepsilon)}\right] + o(\beta), \quad \text{and} \tag{A12}$$

$$\Delta\,{}^{J}\varepsilon = \beta V_{\mathrm{A}}\left(\frac{{}^{J}\tau_{o(J)} - {}^{J}\varepsilon\sum_{i\in J}{}^{i}\varepsilon}{1 + \beta V_{\mathrm{A}}\sum_{i\in J}{}^{i}\varepsilon}\right) + o(\beta), \tag{A13}$$

where ${}^{J}\tau_{o(J)} = \Sigma_k\,{}^{J\cup k}\varepsilon_0\,{}^{k}V_{\mathrm{A}}/V_{\mathrm{A}}$ is a composite representation of directional epistasis of order $o(J)$ acting on the interaction between the loci in the set J.

Appendix 2: Long-Term Rates of Evolution

The asymptotic rate of change in the multilinear model will be determined by the terms of the highest order. Here, we explore this rate in the case of pairwise epistasis. The second-order terms affecting the phenotype can be described by a quadratic form in the reference effects, as $\mathbf{g}^T\mathbf{E}\mathbf{g}/2$, where $\mathbf{g}$ is a column vector of reference effects for the n loci, and $\mathbf{E}$ is a symmetric $n \times n$ matrix of epistasis coefficients with ${}^{ij}\varepsilon$ at the ijth position and zeroes on the main diagonal. To understand the asymptotic rate of change of $\mathbf{g}^T\mathbf{E}\mathbf{g}/2$, we must find an expression for the asymptotic rate of change of the vector of reference effects, $\mathbf{g}$.

Under mutation-limited evolution, each element of $\mathbf{g}$ changes with a rate that is proportional to the expected (positive) size of a new mutation, ${}^{i}m$, times its fixation probability, times the mutation probability per allele, ${}^{i}u$. The fixation probability is approximately $2s$, where s equals the selection gradient, β, multiplied by the effect of a mutation of size ${}^{i}m$ on $\mathbf{g}^T\mathbf{E}\mathbf{g}/2$, which we approximate with ${}^{i}m[d(\mathbf{g}^T\mathbf{E}\mathbf{g})/d\,{}^{i}y]/2$. Thus, averaging over the distribution of positive mutational effects, we get

$$d\,{}^{i}y/dt = \beta\,{}^{i}u\langle {}^{i}m^2\rangle d(\mathbf{g}^T\mathbf{E}\mathbf{g})/d\,{}^{i}y. \tag{A14}$$

In vector notation this is

$$d\mathbf{g}/dt = \beta\mathbf{M}d(\mathbf{g}^T\mathbf{E}\mathbf{g})/d\mathbf{g}, \tag{A15}$$

where $\mathbf{M}$ is a diagonal matrix with the ${}^{i}u\langle {}^{i}m^2\rangle$ as elements on the diagonal. We can compute the derivative as

$$d(\mathbf{g}^T\mathbf{E}\mathbf{g})/d\mathbf{g} = 2\mathbf{E}\mathbf{g}. \tag{A16}$$

Therefore, we obtain the following differential equation for the evolution of y

$$d\mathbf{g}/dt = 2\beta\mathbf{M}\mathbf{E}\mathbf{g}. \tag{A17}$$

Provided $\mathbf{E}$ is nonsingular, the solution to this system is

$$\mathbf{g}(t) = \exp[2\beta\mathbf{M}\mathbf{E}t]\mathbf{g}(0), \tag{A18}$$

where $\mathbf{g}(0)$ is a vector of initial values. We can now investigate what this implies for the evolution of the bilinear form itself. Assume now for simplicity that all loci have the same mutation rates and mutational effect distributions, so that $2\beta\mathbf{M} = k\mathbf{I}$, where k is a constant. Then,

$$\begin{aligned}\mathbf{g}^T(t)\mathbf{E}\mathbf{g}(t) &= \mathbf{g}^T(0)\exp[k\mathbf{E}t]\mathbf{E}\,\exp[k\mathbf{E}t]\mathbf{g}(0) \\ &= \mathbf{g}^T(0)\mathbf{E}\,\exp[2k\mathbf{E}t]\mathbf{g}(0),\end{aligned} \tag{A19}$$

where $\mathbf{g}(0)$ is a vector of intial values for the reference effects (which can be taken to be nonzero, because nonzero starting values would evolve due to the first-order terms we have ignored). We can then diagonalize this expression with an orthogonal transformation, $\mathbf{g} = \mathbf{C}\mathbf{x}$, to get

$$\mathbf{x}^T(t)\mathbf{\Lambda}\mathbf{x}(t) = \mathbf{x}^T(0)\mathbf{\Lambda}\,\exp[2k\mathbf{\Lambda}t]\mathbf{x}(0) = \sum_i x_i^2(0)\lambda_i e^{2k\lambda_i t}, \tag{A20}$$

where $\mathbf{\Lambda}$ is the diagonal matrix with the eigenvalues, λ_i, of $\mathbf{E}$. Because $\mathbf{E}$ is real and symmetric, all eigenvalues are real, and because the trace of $\mathbf{E}$ is zero, at least one eigenvalue must be positive (if there is any epistasis at all).

This shows that the asymptotic dynamics are dominated by $2k$ times the largest positive eigenvalue, $\lambda_{\max}$, of the epistasis matrix (and the contribution of the linear terms are dominated by $k\lambda_{\max}$).