Epistasis and the Mutation Load: A Measurement-Theoretical Approach

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Manuscript received September 9, 2000
Accepted for publication February 15, 2001

ABSTRACT

An approximate solution for the mean fitness in mutation-selection balance with arbitrary order of epistatic interaction is derived. The solution is based on the assumptions of coupling equilibrium and that the interaction effects are multilinear. We find that the effect of m-order epistatic interactions (*i.e.*, interactions among groups of m loci) on the load is dependent on the total genomic mutation rate, U, to the mth power. Thus, higher-order gene interactions are potentially important if U is large and the interaction density among loci is not too low. The solution suggests that synergistic epistasis will decrease the mutation load and that variation in epistatic effects will elevate the load. Both of these results, however, are strictly true only if they refer to epistatic interaction strengths measured in the optimal genotype. If gene interactions are measured at mutation-selection equilibrium, only synergistic interactions among even numbers of genes will reduce the load. Odd-ordered synergistic interactions will then elevate the load. There is no systematic relationship between variation in epistasis and load at equilibrium. We argue that empirical estimates of gene interaction must pay attention to the genetic background in which the effects are measured and that it may be advantageous to refer to average interaction intensities as measured in mutation-selection equilibrium. We derive a simple criterion for the strength of epistasis that is necessary to overcome the twofold disadvantage of sex.

THEORETICAL population genetics is in many ways the biological equivalent of theoretical physics. However, despite its mathematical and statistical sophistication, population genetics differs from the physical sciences in its lack of a theory for measuring its fundamental parameters. Usually, model parameters such as gene effects, selection coefficients, and mutation rates are introduced without consideration of their domain of application, their scale, or how they are to be operationally measured (Wagner and Laubichler 2000). Any population genetical model must be understood as a representation of some simple subset of the genetic system that is embedded in an unspecified genetic and environmental background. The model parameters are then implicitly defined with respect to this background and can only be operationally measured with reference to it. We have previously argued that epistatic gene interactions need to be defined operationally in terms of their dynamical effects (Wagner *et al.* 1998; Hansen and Wagner 2001) and we have developed a model of functional epistasis that makes the role of the genetic background explicit (Hansen and Wagner 2001). In this article we use this model to study the effects of epistasis on the mutation load. We show that an explicit consideration of the "reference genotype" can be a powerful conceptual tool that allows us to describe the relationship between epistasis and the mutation load in a way that is not feasible in standard population genetics theory. We also demonstrate that the reference genotype in which gene effects are measured has important consequences for the dynamical and evolutionary interpretations of epistasis.

Although mutation is a fundamental prerequisite for evolvability, most new mutations are deleterious, and every organism carries a load of deleterious mutations (Haldane 1937; Muller 1950). Estimates of the total genomic mutation rate are still controversial but some estimates indicate that the number of new deleterious mutations may average more than one per individual per generation in animals (Crow and Simmons 1983; Crow 1993; Eyre-Walker and Keightley 1999; Lynch *et al.* 1999). Under mutation-selection balance this translates into a large mutational load, which may have a variety of evolutionary implications. The mutation load may be involved in the evolution and maintenance of recombination and sexual reproduction (Kondrashov 1988), in the evolution of senescence (Rose 1991), in the evolution of reproductive effort late in life (Charlesworth 1990a), in inbreeding depression (Charlesworth *et al.* 1990), and in the evolution of mate choice (Hansen and Price 1999).

Epistasis is fundamentally implicated in many of the evolutionary consequences of mutation load. If deleterious mutations interact synergistically they may be more efficiently removed by selection and the load is decreased (Kimura and Maruyama 1966; Charlesworth 1990b; Gavrilets and deJong 1993). Kondrashov

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(1982, 1984, 1988) argued that this effect might be sufficiently strong to outweigh the twofold disadvantage of sex, and help maintain sexual reproduction. Similarly, synergistic interactions among segregating mutations may enhance the differences in the load between individuals. They may, for example, elevate age and sex differences in genetic fitness with obvious consequences for the evolution of mate choice and life histories (Hansen and Price 1999).

THE MULTILINEAR GENOTYPE-PHENOTYPE MAP

The multilinear model (Hansen and Wagner 2001) was introduced with two aims. One aim was to provide a dynamically tractable representation of functional epistatic interactions, and the other was to ensure that parameters and variables were operationally defined. Toward the second aim we introduced the concept of a reference genotype. This is a real or abstract genotype that serves as a yardstick in which model parameters can be measured. The variables of the model are "reference effects" of genes. The reference effect of a single-locus genotype is defined as the phenotypic effect of substituting this genotype into the reference genotype. Let the variable ${}^{i}y$ represent the reference effects of genotypes at locus i. These variables can assume any real value. We can describe a given genotype, g, in terms of the reference effects of all the loci at which it is (potentially) different from the reference genotype. If there are n such loci, the genotype is described as the set $g = \{{}^{1}y, \ldots, {}^{n}y\}$. A genetic substitution at a locus from ${}^{i}y$ to ${}^{i}y'$ can also be assigned a reference effect as ${}^{i}\delta = {}^{i}y' - {}^{i}y$.

To achieve a tractable representation of functional epistasis we made the assumption that a change in the genetic background can at most lead to a linear transformation of the effects of a gene substitution at a locus. In other words, if a gene substitution has an effect ${}^{i}\delta$ in the reference genotype, then the same gene substitution in another genetic background, g, will be ${}^{g\to i}f\,{}^{i}\delta$, where the epistasis factor ${}^{g\to i}f$, to be defined below, is a constant that depends only on the genetic background g. Thus, all gene substitutions at this locus will be modified by the same factor. Together with the weak, but essential, assumption that the effect of a genotype is independent of the order of substitutions that lead to the genotype, we showed that the map from a genotype g to a univariate trait x can be represented as

$$x = x_0 + \sum_i {}^{i}y + \frac{1}{2}\sum_i\sum_{j\neq i} {}^{ij}\varepsilon\, {}^{i}y\,{}^{j}y + \ldots + \frac{1}{m!}\sum_{J\in P_m} {}^{J}\varepsilon\, {}^{J}y + \ldots, \qquad (1)$$

where x_0 is the value of x in the reference genotype, P_m is the set of all m permutations of loci from g, and ${}^{J}y$ is shorthand for the product ${}^{J}y = \prod_{i\in J} {}^{i}y$. The epistasis coefficient ${}^{ij}\varepsilon$ represents the strength of the interaction between loci i and j. The higher-order epistasis coefficient ${}^{J}\varepsilon$ represents the strength of interaction of loci in the set J. All epistasis coefficients can be measured by substituting appropriate multilocus genotypes into the reference after having measured the single-locus reference effects. From this we observe that epistasis coefficients are also defined relative to a reference genotype.

The reference effects are defined with respect to whole-locus genotypes only. Substitution of single alleles is a special case of this, and the theory makes no assumptions about dominance. The linearity assumption, however, implies that additive and dominance effects are combined in the same fashion. Hence, there is no distinction among A × A, A × D, and D × D epistasis.

By the "genetic background" of a single- or multilocus genotype we mean the state of all other loci in g. The effect of a genetic background, g, on a single-locus genotype, i, can be described in terms of an epistasis factor

$${}^{g\to i}f = 1 + \sum_{j\neq i} {}^{ij}\varepsilon\, {}^{j}y + \ldots + \frac{1}{m!}\sum_{J\in P_m(g\setminus i)} {}^{i\cup J}\varepsilon\, {}^{J}y, \qquad (2)$$

where $P_m(g\setminus i)$ is the set of m permutations from the set $g\setminus i$ (*i.e.*, g except i), such that the effect of a substitution at locus i with reference effect ${}^{i}\delta$ has effect ${}^{g\to i}f\,{}^{i}\delta$ in the background of g. Higher-order epistasis factors describing the effect of a background on interactions among loci can also be defined. Specifically, let J be a set of indices representing the loci at which a substitution has occurred, such that ${}^{J}\delta$ is the product of the reference effects of all loci in J and ${}^{J}\varepsilon$ is their epistasis coefficient. The epistasis term describing the interaction among these loci is ${}^{J}\varepsilon{}^{J}\delta$, and in the background of g this term is modified into ${}^{g\to J}f\,{}^{J}\varepsilon{}^{J}\delta$, by the epistasis factor

$${}^{g\to J}f = \frac{\sum_{K\in\wp(g\setminus J)} {}^{J\cup K}\varepsilon\, {}^{K}y}{{}^{J}\varepsilon}, \qquad (3)$$

where the K's are sets of indices running over all possible unordered subsets of indices from g excluding J [*i.e.*, the power set $\wp(g\setminus J)$]. The index set $J\cup K$ is the union of indices from J and K, and it is understood that ${}^{K}y = 1$ when K is empty.

If the reference genotype is changed, say from a genotype r to a genotype r', the reference effects and epistasis coefficients measured in r' are

$${}^{i}y' = {}^{r'\to i}f({}^{i}y - {}^{i}\Delta), \quad {}^{J}\varepsilon' = \frac{{}^{r'\to J}f\,{}^{J}\varepsilon}{\prod_{j\in J} {}^{r'\to J}f}, \qquad (4)$$

where all entities on the right-hand side are measured with reference to r, and ${}^{i}\Delta$ is the reference effect of the substitution at locus i that separates r' from r.

Proofs and further details are given in Hansen and Wagner (2001). A further result that becomes important below, and is proven in appendix a, is that the multilinear model can also be written in terms of epistasis factors as

$$x = x_0 + \sum_i {}^{g\to i}f\,{}^i y - \frac{1}{2}\sum_i\sum_{j\neq i} {}^{g\to ij}f\,{}^{ij}\varepsilon\,{}^i y\,{}^j y - \ldots - \frac{(-1)^m}{m!}\sum_{J\in P_m} {}^{g\to J}f\,{}^J\varepsilon\,{}^J y + \ldots . \quad (5)$$

THE MUTATION LOAD

Let w be fitness with optimal (= maximal) value w_0. Further, let the genetic architecture of fitness be described as a multilinear form in the reference effects of n diallelic loci. We start by using the optimal genotype as reference.

Let ${}^i a$ be the deleterious reference effect of a heterozygote at locus i. Technically this reference effect is then defined on a negative fitness scale (*i.e.*, the reference effect is $-{}^i a$ on the fitness scale). This is convenient as it allows us to represent deleterious effects as positive numbers. Let ${}^i p$ be the frequency of the deleterious nonrecessive allele at locus i, and let its mutation rate per allele per generation be ${}^i u$. The mean deleterious reference effect of the locus is then ${}^i\bar{y} \cong 2{}^i a\,{}^i p$. Observe that the actual fitness effect of the heterozygote in a given genetic background, g, is $(-{}^{g\to i}f\,{}^i a)$. Using this, and assuming all loci are in coupling equilibrium and ignoring back mutations, we have

$$\Delta {}^i p \cong -{}^{\bar{g}\to i}f\,{}^i a\,{}^i p\,{}^i q + {}^i u\,{}^i q, \quad (6)$$

where ${}^i q = 1 - {}^i p$, and $\bar{g} = \{{}^1\bar{y}, \ldots, {}^n\bar{y}\}$ is a genotype in which each locus has the reference effect equal to the population average. In mutation-selection balance we get

$$\hat{\bar{g}}\to i f\,{}^i a\,{}^i\hat{p} = {}^i u, \quad (7)$$

$$\hat{\bar{g}}\to i f\,{}^i\hat{\bar{y}} = 2{}^i u. \quad (8)$$

A carat (^) above a symbol is used to denote equilibrium value. In APPENDIX B we show that the exact same relationship holds also when there are multiple deleterious alleles at the locus provided ${}^i u$ is interpreted as the total deleterious mutation rate at the locus. A sufficient condition for local stability of the equilibrium is also provided in APPENDIX B.

Again assuming coupling equilibrium, the average fitness is

$$\bar{w} = w_0 - \Big(\sum_i {}^i\bar{y} + \frac{1}{2}\sum_i\sum_{j\neq i} {}^{ij}\varepsilon\,{}^i\bar{y}\,{}^j\bar{y} + \ldots + \frac{1}{m!}\sum_{J\in P_m} {}^J\varepsilon\,{}^J\bar{y} + \ldots\Big). \quad (9)$$

Note that a positive epistasis coefficient describes enhancement of the deleterious effects at a group of loci. Thus, positive epistasis coefficients represent synergistic epistasis among deleterious mutations and negative epistasis coefficients represent antagonistic epistasis.

The genetic load is $L = w_0 - \hat{\bar{w}}$. Rewriting (9) as a load, and then using the expansion into epistasis factors (*i.e.*, Equation 5), we get

$$L = \sum_i {}^{\hat{\bar{g}}\to i}f\,{}^i\hat{\bar{y}} - \frac{1}{2}\sum_i\sum_{j\neq i} {}^{\hat{\bar{g}}\to ij}f\,{}^{ij}\varepsilon\,{}^i\hat{\bar{y}}\,{}^j\hat{\bar{y}} - \ldots - \frac{(-1)^m}{m!}\sum_{J\in P_m} {}^{\hat{\bar{g}}\to J}f\,{}^J\varepsilon\,{}^J\hat{\bar{y}} - \ldots . \quad (10)$$

Using (8) then gives

$$L = 2\sum_i {}^i u - 2\sum_i\sum_{j\neq i}\frac{{}^{\hat{\bar{g}}\to ij}f\,{}^{ij}\varepsilon\,{}^i u\,{}^j u}{{}^{\hat{\bar{g}}\to i}f\,{}^{\hat{\bar{g}}\to j}f} - \ldots - \frac{(-2)^m}{m!}\sum_{J\in P_m}\frac{{}^{\hat{\bar{g}}\to J}f\,{}^J\varepsilon\,{}^J u}{\prod_{j\in J}{}^{\hat{\bar{g}}\to j}f} - \ldots , \quad (11)$$

where ${}^J u = \prod_{j\in J}{}^j u$. This solution is not complete since the epistasis factors are functions of the equilibrium gene frequencies. However, using (4), we may express the solution in terms of epistasis coefficients, ${}^J\varepsilon'$, measured with reference to the background $\hat{\bar{g}}$, as

$$L = 2\sum_i {}^i u - 2\sum_i\sum_{j\neq i} {}^{ij}\varepsilon'\,{}^i u\,{}^j u - \ldots - \frac{(-2)^m}{m!}\sum_{J\in P_m} {}^J\varepsilon'\,{}^J u - \ldots . \quad (12)$$

Note that no other parameters need to be changed as mutation rates are invariant to choice of reference genotype. Provided coupling disequilibrium can be ignored, this equation constitutes an approximate solution to the mutation load with multilinear epistatic interactions of arbitrary order.

Equation 12 does not actually predict the equilibrium mean fitness given *a priori* mutation rates and other genetics parameters. The reason is that the epistasis coefficients in (12) are defined and measured at mutation-selection equilibrium and are thus strictly spoken functions of the model variables rather than constant parameters. However, there is an interpretation of this equation that is nevertheless useful. The first term, $2\Sigma_i {}^i u = U$, where U is the total genomic deleterious mutation rate, is the genetic load of a strictly additive system in mutation-selection equilibrium. This term is invariant to the change in reference genotype that allowed us to derive this equation. The equation thus predicts to what extent the mean fitness of a population with interaction effects is different from that of an additive model. The degree of deviation from the additive prediction is determined by the strength of interaction as measured in the equilibrium population. This has the advantage that the relevant epistatic interaction strength is operationally defined in the population rather than with reference to some usually unknown "optimal" genotype. Hence the relevant strength of interaction in this formulation is actually measurable, while in the original formulation it may not be, unless the optimal genotype is available for experimentation.

A further result, which is established in APPENDIX C, is that if $w({}^1y', \ldots, {}^ny')$ is the fitness of a genotype as

measured in the equilibrium, then the fitness of the optimal genotype is

$$w_0 = w(-2^1 u, \ldots, -2^n u). \quad (13)$$

This result is useful for computing the load of a given fitness function. For example, if fitness is multiplicative at equilibrium, $w = \Pi_i(1 - {}^i y')$, then the optimal genotype has fitness

$$w_0 = \prod_i (1 + 2^i u) = \mathrm{Exp}[U]. \quad (14)$$

As the mean of ${}^i y'$ is zero, the mean fitness is one. Thus, we have established the classical result that the ratio of mean to maximum fitness is $\mathrm{Exp}[-U]$ under multiplicative epistasis (*e.g.*, Charlesworth 1990b).

EFFECTS OF EPISTASIS ON THE MUTATION LOAD

The first term in (11) and (12) corresponds to the classical result (Haldane 1937) $L = U$ for the mutation load of a trait with an additive genetic basis. If epistasis is present this can be modified in various directions. To see the effects more transparently, we can rewrite (12) as

$$L = U - \frac{1}{2}{}^{(2)}\varepsilon' U^2 - \ldots - \frac{(-1)^m}{m!}{}^{(m)}\varepsilon' U^m - \ldots, \quad (15)$$

where ${}^{(m)}\varepsilon' = \Sigma_{J \in P_m^J} \varepsilon' \prod_{j \in J} (2^j u / U)$ is an effective epistasis coefficient of order m, which can be interpreted as a measure of the average direction of mth-order epistasis. The second term in this equation shows that synergistic pairwise epistasis reduces the mutation load in a sexual population. This is consistent with the results of Kimura and Maruyama (1966) and Charlesworth (1990b) who, on the basis of a model where fitness was a quadratic function of number of deleterious alleles in the genotype, showed that synergistic interactions would reduce the load. This effect is proportional to the square of U, and may be substantial if U is substantially >1 (Kondrashov 1988).

A novel result of some interest is that higher-order epistasis potentially may have very strong effects on the load if U is large. This is because the effect of mth-order epistasis is proportional to U^m. Of course, this interpretation depends critically on the effective epistasis coefficients of higher order not being vanishingly small. There are several reasons why coefficients of higher order may be expected to be smaller. First, note that the effective epistasis coefficients are strict averages of the individual epistasis coefficients only if all loci have equal mutation rates and the number of loci is much larger than the order of the interaction. The second of these caveats is unlikely to be important as fitness components are usually influenced by many loci. However, unequal mutation rates may reduce higher-order effective coefficients well below the average interaction strength. Second, functional epistatic interactions among many loci may be very infrequent, making the effective epistasis coefficients near zero. Nevertheless, provided the interaction density is not too low, we suggest that higher-order interactions may strongly influence the load when U is large.

A surprising result is that the sign of the effects of synergistic epistasis seems to depend on whether the interaction is among an odd or even number of genes. The opposite effects of even- and odd-order synergistic interactions on genetic load need careful interpretation. If we start with (11) describing the solution when effects are measured in the optimal genotype, we can observe that there are two effects of epistasis of a given order. One is given directly and the other is given as a modification of lower-order effects through the epistasis factors. By differentiation of (11) it can be shown that increasing any epistasis coefficient will lead to a reduction in the load provided all the single-locus epistasis factors are relatively close to one. Thus, at least for weak epistasis, synergistic interactions of any order will reduce the load.

However, as can be seen from (12) and (15), odd-order synergistic epistasis as measured with reference to the equilibrium population always elevates the load. To understand how this is possible we may consider what will happen if we take a population where both second- and third-order epistasis are synergistic with reference to the optimal genotype and then measure the interaction strengths in the equilibrium population. What we will observe is that second-order interactions will be much stronger (relative to a population without synergistic third-order interactions; synergistic pairwise epistasis will in fact be weaker at equilibrium if there are no higher-order interactions), while third-order interactions will be weaker. Thus, the two results are consistent. This underscores the importance of knowing exactly in what sort of genetic background epistatic effects are measured.

Effects of variation in epistasis: It has been argued that variation in epistatic interactions across loci will elevate the load and make the evolution of recombination less favorable (Otto and Feldman 1997; Phillips *et al.* 2000). As gene interaction is likely to be extremely variable, this is a potentially fatal argument against the hypothesis that recombination is an adaptation to reduce the mutation load. These results were based on the analysis of two-locus models, and it is of interest to see how they hold up in a multilocus analysis.

The effect may be seen most clearly with second-order epistasis measured with reference to the optimum genotype [see (11)]. Observe that the epistasis coefficients are weighted with the inverse of single-locus epistasis factors. If there is a lot of variation in epistatic interactions, the epistasis factors will be variable. Some will be larger than one and some will be smaller than one. If a locus has a lot of antagonistic epistatic interactions with other loci, the epistasis factor acting on this locus will tend to become less than unity. This will en-

hance the effect of all interactions involving this locus. Therefore, the load-elevating effects of antagonistic epistasis will tend to be enhanced by variation in epistatic interactions. Similarly, the load-reducing effects of synergistic epistasis will be diminished, and we predict that variation in pairwise epistasis will increase the load.

However, this argument pertains to epistatic interactions measured with reference to the optimum genotype. Variation in epistasis measured in the equilibrium genotype has no inherent tendency to elevate the load [see (12)]. For this reason, there are heuristic advantages to measuring directional epistasis in the average genotype, as this can be used to assess its effects on the load in an unbiased fashion.

THE AMOUNT OF GENE INTERACTION NECESSARY TO MAINTAIN SEX

Given (15) one may ask how strong epistasis needs to be to compensate for the twofold advantage of asexual reproduction. Let $W(N)$ be the per capita growth rate of a mutation-free sexual population as a function of population density, N. All else being equal we expect the growth rate of a mutation-free asexual clone to be $2W(N)$. Assume that the deleterious mutations have the same effects on fitness at all densities. In mutation-selection equilibrium the mean fitness of a sexual population will then be

$$W_{\text{sex}} = W(N) - U + \frac{1}{2}{}^{(2)}\varepsilon' U^2 + \ldots, \qquad (16)$$

and that of the asexual clone,

$$W_{\text{clone}} = 2W(N) - U. \qquad (17)$$

If we assume only pairwise epistasis, it is easy to see from these equations that $W_{\text{sex}} \geq W_{\text{clone}}$ if

$${}^{(2)}\varepsilon' \geq \frac{2W(N)}{U^2}. \qquad (18)$$

In a sexual population at equilibrium density, $W(\hat{N}) = 1 + U - 1/2{}^{(2)}\varepsilon' U^2$, and using this we can show that the population can resist invasion from an asexual clone if

$${}^{(2)}\varepsilon' \geq \frac{1 + U}{U^2}, \qquad (19)$$

and it can be shown that the exact same criterion allows a sexual population to invade an asexual clone at its equilibrium density.

This implies that sex is likely to be maintained by synergistic epistasis only if the total genomic mutation rate is large. Given estimates of U and of directional epistasis, (19) provides a test criterion that can be used to evaluate whether synergistic epistasis could be strong enough to maintain sex. To interpret this result it is useful to observe that an epistasis coefficient equal to 2, which would be necessary if $U = 1$, means that an average deleterious mutation with a 1% effect on fitness would increase the effects of other mutations at least 2% on average. Given that not all mutations affect each other, this must be considered strong epistasis. If $U = 10$, a mutation with a 1% effect needs to increase the effect of other mutations at least 0.11% on average.

We add the caveat that the above analysis presupposes that both sexuals and asexuals are in mutation-selection equilibrium when they start to compete. If an asexual clone arises directly from the sexual population itself, it may be able to invade before it has acquired an elevated load. Thus, complicated dynamics may easily ensue, where asexuals first spread and then get outcompeted as their loads increase.

Equation 18 indicates that sex is most likely to be favored when the per capita growth rate is low. If competition between sexual and asexual individuals is limited to periods of rapid population growth, as may happen in an r-selected species, sexual reproduction will be more difficult to maintain.

In conclusion, sex is likely to be favored if the genome is large (*i.e.*, U is large) and the growth rate is low. Assuming that there is no intrinsic difference in the average degree of gene interaction between organisms with small genomes and those with large genomes, sex is expected to be more readily maintained in slowly reproducing organisms with many genes. This is consistent with a number of natural history facts. For instance, the frequency of asexually reproducing species is lower among higher animals with large genomes and slow reproduction. Another pattern is the prevalence of parthenogenetic species in pioneer species that are characterized by high reproductive rates. We note, however, that there are other hypotheses that can account for these differences (Maynard Smith 1976; Bell 1982).

THE FIXATION LOAD

Another consequence of synergistic epistasis is its potential ability to halt Muller's ratchet in asexuals (Kondrashov 1994). However, this may be unlikely as it depends critically on the unrealistic assumption that the effects of all mutations are the same (Butcher 1995). Here, we suggest another potentially important role for epistasis in the fixation of deleterious mutations.

We suggest that synergistic epistatic interactions tend to become reduced and antagonistic interactions elevated as we move away from the optimum genotype. This comes about because the effect of a new deleterious mutation in a background g is ${}^{g\rightarrow ij}\delta$. Due to the fact that the epistasis coefficients that determine the epistasis factor are symmetrical across loci (${}^{ij}\varepsilon = {}^{ji}\varepsilon$, and so on), this means that a mutation on a locus with a lot of antagonistic interactions with other loci will tend to have reduced effect due to segregation or fixation of mutations on other loci. Therefore, its fixation probabil-

ity will be increased. For the same reason, a mutation with a lot of synergistic interactions will have reduced fixation probability. Due to the fact that the fixation probability of deleterious mutations is an extremely nonlinear function of the effect of these mutations (Bürger and Ewens 1995), this may lead to extreme differences in the fixation probabilities of mutations with different sorts of epistatic interactions. The ratchet clicks faster with antagonistic epistasis.

The implication of this is that the process of fixing deleterious mutations may be associated with the evolution of antagonistic epistatic interactions, and this will be true for epistasis of all orders. If there are suites of mutations with mutually antagonistic interaction (*e.g.*, compensatory mutations), then the fixation, or even segregation, of some of these will alter the genetic background in a way that may greatly elevate the fixation probability of the other mutations. Thus, in small populations, we may expect a genetic architecture where many alleles have strong deleterious effects with reference to an optimal or ancestral genotype, but where antagonistic epistasis keeps the fixation load bounded. Note also that, in such a population, the individual epistatic interactions as measured in the optimal genotype do not have to be strong. Thus, it is essential to measure epistatic interactions with reference to the population in which they occur. The effect of such a process on the evolution of recombination needs investigation.

DISCUSSION AND CONCLUSION

Epistasis is an evolving entity. The phenotypic effects of gene interactions depend on the genetic background in which the interactions take place and will change when the genetic background is changing. This has also been found in empirical studies (Moreno 1994; Polaczyk *et al.* 1998). Therefore, both empirical and theoretical studies should pay close attention to the genetic background in which epistasis is measured. The concept of a reference genotype makes this explicit, and has led to several new insights about the effects of gene interaction on the mutation load.

What do the results imply for the maintenance of sex and recombination? The most important implication is that the pattern of higher-order epistasis may be important. The deterministic-mutation hypothesis (Kondrashov 1988) depends on a large value of the total deleterious mutation rate, U, and this is precisely the situation in which higher-order interactions start to affect the load. How large U needs to be before higher-order interactions become important depends critically on the relative strength of higher-order epistasis. Although difficult, the estimation of higher-order epistasis coefficients may be helpful to understand the role of epistasis in the maintenance of sex and recombination.

The notion that synergistic epistasis is reducing the load in a sexually reproducing population has also been refined. We have confirmed the insight that the mutation load is reduced by synergistic epistasis, at least as long as the epistatic effects are not very strong. However, this is strictly true only if the statement refers to gene interactions measured in the optimal genotype. As measured in the equilibrium population, synergistic interactions among odd-numbered genes will in fact always elevate the load. Note, though, that this may then be compensated by a relative increase in even-ordered interaction strengths.

The asymmetry between odd- and even-ordered epistasis is indeed a puzzling result. One interpretation (suggested by J. Hermisson, personal communication) is that it may be due to an asymmetric effect of even- and odd-ordered epistasis on beneficial alleles. At equilibrium, a synergistic interaction among an even number of deleterious alleles implies an antagonistic interaction among alleles that are beneficial relative to the mean. However, a synergistic interaction among an odd number of deleterious alleles implies a synergistic interaction among beneficial alleles. It is possible that this asymmetry can explain the different effects on the load of even- and odd-order interactions as measured in the "average genotype."

Variation in pairwise epistatic interactions may indeed be said to elevate the load if the statement refers to pairwise epistasis measured with reference to the optimal genotype (as in Otto and Feldman 1997; Phillips *et al.* 2000). However, we found no obvious relationship between the size of the load and variation in epistasis as measured in the equilibrium population.

Epistatic interactions have been measured in a number of studies including quantitative trait loci data (*e.g.*, Cheverud 2001) or in genotypes in which mutations have been induced or been allowed to accumulate Mukai 1969; Clark and Wang 1997; Whitlock and Bourguet 2000 in Drosophila; De Visser *et al.* 1996, 1997a in Chlamydomonas; De Visser *et al.* 1997b in Aspergillus; Elena and Lenski 1997 in *Escherichia coli*). Before such estimates can be related to the mutation load and used to test evolutionary hypotheses, the genetic background in which they are measured should be precisely characterized. If the genetic background can be assumed to have reached mutation-selection equilibrium, then the effects of induced mutations can be interpreted with our equilibrium reference theory, and their impact on the load and the advantage of recombination can be evaluated by (15) and (19).

Unfortunately, with the possible exception of Elena and Lenski's (1997) study of asexual prokaryotes, no existing studies can be said to measure gene interactions with reference to an equilibrium background or to an optimal background. There is a need for measures of epistasis in wild-type backgrounds, or at least in backgrounds where the fitness effects of the genetic differences to a wild-type or optimal genotype are known, so that the effects of a change in reference can be assessed.

The most useful measures of epistasis may very well be those that are made in natural equilibrium populations or at least in lab populations that can be assumed to have reached mutation-selection balance in a stable environment. This is because the effects on the load are then not obscured by variation in epistatic effects, and because this is the situation in which evolution actually takes place. It is therefore encouraging that large-scale studies of epistasis in natural populations are now emerging (*e.g.*, Fenster and Galloway 2000).

The authors are grateful to Reinhard Bürger, David Houle, Alex Kondrashov, and an anonymous reviewer for helpful comments on previous versions of the manuscript; and to Joachim Hermisson for many stimulating discussions and helpful insights that improved the manuscript. A postdoctoral fellowship from the Norwegian science council supported T.F.H.

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Communicating editor: M. K. Uyenoyama

APPENDIX A

Result: The genotypic value for a trait under multilinear epistasis in genotype g can be written as

$$x = x_0 + \sum_i {}^{g\to i}f^i y - \frac{1}{2}\sum_i\sum_{j\neq i} {}^{g\to ij}f^{ij}\varepsilon^i y^j y - \ldots - \frac{(-1)^m}{m!}\sum_{J\in P_m} {}^{g\to J}f^J\varepsilon^J y \ldots, \quad \text{(A1)}$$

where P_m is the set of all m permutations of loci from g, and the mth order epistasis factor is defined as

$${}^{g\to J}f = \frac{\sum_{K\in\wp(g\setminus J)} {}^{J\cup K}\varepsilon^K y}{{}^J\varepsilon}, \quad \text{(A2)}$$

where $\wp(g\setminus J)$ is the power set of all loci in g except those in J.

Proof: We need to show that the formula is equivalent to the multilinear model,

$$x = x_0 + \sum_i {}^i y + \frac{1}{2}\sum_i \sum_{j\neq i} {}^{ij}\varepsilon\, {}^i y\, {}^j y + \ldots + \frac{1}{m!}\sum_{J\in P_m} {}^J\varepsilon\, {}^J y + \ldots . \tag{A3}$$

As the two equations contain exactly the same types of terms, it suffices to show that the coefficients for the terms match in the two formulas. Let the kth-order set of terms in the epistasis-factor expansion be written

$$a_k \sum_{J\in C_k} {}^{g\to J}f\, {}^J\varepsilon\, {}^J y = a_k \sum_{J\in C_k} {}^J\varepsilon\, {}^J y \left(\sum_{R\in \wp(J)} \frac{{}^{R\cup J}\varepsilon\, {}^R y}{{}^J\varepsilon} \right), \tag{A4}$$

where C_k is the set of all k combinations from g, and a_k is the coefficient pertaining to this term. By hypothesis $a_k = (-1)^{k+1}$, and we proceed to verify that this indeed makes the two equations equal. We can write (A4) as a sum over terms of order m,

$$a_k \sum_{m\geq k} \sum_{J\in C_k} \sum_{C_{m-k}(g\setminus J)} {}^{R\cup J}\varepsilon\, {}^J y\, {}^R y, \tag{A5}$$

where $C_{m-k}(g\setminus J)$ is the set of all $(m-k)$ combinations from the set $g\setminus J$. Changing this from combinations to permutations we get

$$a_k \sum_{m\geq k} \sum_{J\in P_k} \sum_{P_{m-k}(g\setminus J)} \frac{{}^{R\cup J}\varepsilon\, {}^J y\, {}^R y}{k!\,(m-k)!} = a_k \sum_{m\geq k} \sum_{J\in P_m} \frac{{}^J\varepsilon\, {}^J y}{k!\,(m-k)!}, \tag{A6}$$

where $P_{m-k}(g\setminus J)$ is the set of all $(m-k)$ permutations from the set $g\setminus J$. We can now compare this to the corresponding term in the multilinear model:

$$\sum_{k\leq m} a_k \sum_{J\in P_m} \frac{{}^J\varepsilon\, {}^J y}{k!\,(m-k)!} = \frac{1}{m!}\sum_{J\in P_m} {}^J\varepsilon\, {}^J y. \tag{A7}$$

This leads to the equation

$$\frac{1}{m!} = \sum_{k=1}^{m} \frac{a_k}{k!\,(m-k)!}, \tag{A8}$$

which can be written in the form

$$a_m = 1 - \sum_{k=1}^{m-1} \binom{m}{k} a_k, \tag{A9}$$

by hypothesis $a_k = (-1)^{k+1}$, from which it is easily verified that $a_m = (-1)^{m+1}$ for all m. QED

APPENDIX B

If there are multiple alleles segregating at a locus we can derive (8) in the following way. Let ${}^i q$ be the frequency of the optimal allele at locus i, and let ${}^i\overline{a}$ be the average deleterious reference effect of the locus when a deleterious allele is present. The mean reference effect at the locus is then ${}^i\overline{y} = 2\,{}^i\overline{a}(1 - {}^i q)$. Let ${}^i x$ be an indicator variable that takes the value 1 if a random allele at locus i is the optimal allele and 0 otherwise. The expectation of ${}^i x$ is then ${}^i q$. The Price equation then gives

$$\Delta^i q = \Delta\langle {}^i x\rangle = \mathrm{Cov}[w, {}^i x] + \langle \Delta^i x\rangle \approx -\,{}^{\bar g\to i}f\,\mathrm{Cov}[{}^i y, {}^i x] - {}^i u\, {}^i q$$
$$\approx -\,{}^{\bar g\to i}f\left(\frac{1}{2}\,{}^i\overline{y}\, {}^i q - {}^i\overline{y}\, {}^i q\right) - {}^i u\, {}^i q = \frac{1}{2}\,{}^{\bar g\to i}f\, {}^i\overline{y}\, {}^i q - {}^i u\, {}^i q. \tag{B1}$$

Here the variable ${}^i y$ is assumed to take the value ${}^i\overline{y}/2$ if ${}^i x = 1$ and ${}^i\overline{a}$ if ${}^i x = 0$. The second term in the equation represents the change in ${}^i q$ due to mutation. Thus, ${}^i u$ is the probability per allele of mutating away from the optimal state. With small mutation rates this will equal the sum of the probabilities of all possible deleterious mutations at the locus. We ignore back mutation. Thus, (8) is valid for multiple alleles provided ${}^i u$ is interpreted as the total deleterious mutation rate at the locus.

The local stability of the equilibrium reference effects can be investigated through the Jacobian matrix for the system of equations

$$\Delta^i\overline{y} = -2\,{}^i\overline{a}\,\Delta^i q = ({}^i\overline{a} - {}^i\overline{y}/2)(2\,{}^i u - {}^{\bar g\to i}f\, {}^i\overline{y}), \tag{B2}$$

which is derived from (B1) by using ${}^i\overline{y} = 2\,{}^i\overline{a}(1 - {}^i q)$. Differentiating and evaluating at the equilibrium then gives $1 - {}^{\hat{\bar g}\to i}f\,({}^i\overline{a} - {}^i\hat{\overline{y}}/2)$ for the ith diagonal element of the Jacobian and $-{}^{\hat{\bar g}\to ij}f\, {}^{ij}\varepsilon\, {}^i\hat{\overline{y}}({}^i\overline{a} - {}^i\hat{\overline{y}}/2)$ for the ijth off-diagonal element. To obtain this, it helps to note that $\partial {}^{g\to i}f/\partial {}^j y = {}^{g\to ij}f\, {}^{ij}\varepsilon$.

Stability requires that all eigenvalues of the Jacobian are inside the unit circle in the complex plane. Note first that, as long as the epistasis factors are positive, the diagonal elements are always less than one. Gerschgorin's theorem says that all eigenvalues must be inside the union of the disks centered at the diagonal elements with radii equal to the sum of the absolute values of the off-diagonal elements in the corresponding row. Thus, a sufficient condition for local stability is that the following relations hold for all loci, i,

$$\begin{aligned} &{}^{\hat{\bar g}\to i}f > 0 \\ &{}^{\hat{\bar g}\to i}f^2 > 2\,{}^i u \sum_j |{}^{\hat{\bar g}\to ij}f\, {}^{ij}\varepsilon| \\ &2\,{}^{\hat{\bar g}\to i}f > ({}^i\overline{a} - {}^i\hat{\overline{y}}/2)({}^{\hat{\bar g}\to i}f^2 + 2\,{}^i u \sum_j |{}^{\hat{\bar g}\to ij}f\, {}^{ij}\varepsilon|). \end{aligned} \tag{B3}$$

This criterion shows that the equilibrium is stable as long as epistasis and the deleterious heterozygote effects of mutations are not extremely strong. It also indicates that synergistic epistasis tends to promote stability, as the epistasis factors on the left-hand sides in (B3) are then >1. Antagonistic epistasis reduces the epistasis factors, and, for mutations with small effects, an unstable equilibrium only seems plausible in situations involving strong antagonistic epistasis.

APPENDIX C

Result: If $w({}^1y', \ldots, {}^ny')$ is the fitness of a genotype as measured in mutation-selection equilibrium, then the fitness of the optimal genotype is

$$w_0 = w(-2^1u, \ldots, -2^nu). \quad \text{(C1)}$$

Proof: When we use the mutation-selection equilibrium genotype as reference genotype, the mean reference effects are 0 at all loci. Thus the mean fitness is $w(0, \ldots, 0)$. To find the fitness of the optimal genotype, all we need to do is to find the reference effects of the optimal genotypes at each locus and substitute into $w({}^1y', \ldots, {}^ny')$.

Obviously, the reference effects of the single-locus optimal genotypes are 0 when measured in the optimal genotype. We use this as a starting point, and apply the change of reference formula (4) to compute the reference effects measured at equilibrium.

$$\begin{aligned} {}^iy' &= {}^{\hat{\bar{g}}\to i}f\,({}^iy - {}^i\Delta) = {}^{\hat{\bar{g}}\to i}f\,(0 - 2^iu/{}^{\hat{\bar{g}}\to i}f) \\ &= -2^iu. \end{aligned} \quad \text{(C2)}$$

The variable ${}^i\Delta$ is the effect of the new reference as measured in the old reference. Thus, in (C2), ${}^i\Delta$ is the mean effect at the locus as measured in optimal genotype. This is given in (8) as ${}^i\hat{\bar{y}} = 2^iu/{}^{\hat{\bar{g}}\to i}f$.

QED