Interaction between directional epistasis and average mutational effects

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We investigate the relationship between the average fitness decay due to single mutations and the strength of epistatic interactions in genetic sequences. We observe that epistatic interactions between mutations are correlated to the average fitness decay, both in RNA secondary structure prediction as well as in digital organisms replicating *in silico*. This correlation implies that, during adaptation, epistasis and average mutational effect cannot be optimized independently. In experiments with RNA sequences evolving on a neutral network, the selective pressure to decrease the mutational load then leads to a reduction in the amount of sequences with strong antagonistic interactions between deleterious mutations in the population.

Keywords: epistasis; neutrality; RNA secondary structure folding; digital organisms

1. INTRODUCTION

A thorough understanding of epistatic interactions of mutations in genomes is becoming more and more crucial to many areas in population genetics and evolutionary biology. Epistasis affects linkage disequilibria (Charlesworth 1976; Barton 1995), robustness to mutations (Lenski *et al*. 1999) or canalization (Nowak *et al*. 1997; Wagner *et al*. 1997; Rice 1998; Ancel & Fontana 2000; for reviews, see Scharloo 1991; Gibson & Wagner 2000), as well as theories on the maintenance of sex (Kondrashov 1982, 1988; West *et al*. 1999). The sign of epistatic effects, that is whether deleterious mutations are reinforcing (synergistic epistasis) or mitigating (antagonistic epistasis), also influences whether or not deleterious mutations can accumulate in the genome via Muller's ratchet (Muller 1964; Felsenstein 1974). The consensus seems to be that synergistic epistasis can prevent the accumulation of mutations (Crow & Kimura 1978; Kondrashov 1994, but see Butcher (1995) for a dissenting view). On the other hand, the observation of pervasive compensatory mutations (Moore *et al*. 2000), which also render the ratchet powerless, indicates epistasis but not its sign.

While the genomes of a number of organisms have been examined for signs of epistasis (De Visser *et al*. 1997*a,b*; Elena & Lenski 1997; De Visser & Hoekstra 1998; Elena 1999), no general trend can be discerned except to say that interactions between mutations are frequent and of both signs, and that weak synergistic epistasis seems to prevail in eukaryotic genomes while viral and prokaryotic genomes show no net tendency in either direction. Experiments for measuring epistatic interactions are difficult and usually yield results of weak statistical significance (West *et al*. 1998). Consequently, even epistasis of considerable strength can conceivably be missed *in vitro* or *in vivo*. Here, we investigate deleterious mutations *in silico* and study a fact that has not received much attention in the population genetics literature, namely that epistasis is closely related to the geometry of the phenotype space (Rice 1998), which leads to

interesting relations between epistasis and the effects of single mutations. Wagner *et al*. (1998) showed in a two-locus, two-allele model that the average effect of a single mutation is correlated to the degree of interaction between loci and supported their theoretical model with quantitative trait locus data on body weight in mice. Here we demonstrate a similar relation for a measure of epistasis that is more appropriate for high-dimensional sequence spaces. We give both theoretical and experimental evidence that the strength of directional epistasis is correlated with the average deleterious effect of a single mutation. As a corollary of this observation, we argue that, in situations in which there is selective pressure to reduce the average deleterious effect, this correlation leads to a reduction in the number of genomes with strong antagonistic effects in a population.

2. NEUTRALITY AND EPISTASIS

It is a common observation that the average fitness at a mutational distance n from a given reference sequence decays approximately exponentially with n (see, for example, De Visser *et al*. 1997*b*; Elena & Lenski 1997; Lenski *et al*. 1999) (see also our results on RNA sequences below). The simplest explanation for a perfect exponential is a multiplicative landscape in which every mutation diminishes the fitness independently by the same factor $(1-s)$. Here, however, we focus on fitness landscapes with a considerable amount of lethal mutations, in which case a branching process in the high-dimensional sequence space is a more appropriate model: if every viable sequence has a probability of $1-s$ for remaining viable after suffering a single-point mutation, then the mean fitness will decay as (neglecting fitness differences in the viable sequences)

$$w(n) = (1-s)^n \equiv e^{-\alpha n}, \qquad (2.1)$$

where we have defined $\alpha = -\ln(1-s)$ and assumed that the fitness of our reference sequence at $n=0$ is $w(0)=1$. Both explanations for the exponential decay have in common the fact that the effects of subsequent mutations

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are independent, i.e. there exists no epistasis. If there are some interactions between mutations, we will still observe an exponential decay if antagonistic and synergistic interactions occur in the same proportions. However, if a bias towards either antagonistic or synergistic epistatic interactions exists (directional epistasis), then this bias will naturally appear as deviations from the exponential decay in equation (2.1). While such deviations have previously been indicated by adding a term quadratic in n to the exponent of equation (2.1) (Crow 1970; Charlesworth 1990; De Visser *et al.* 1997*b*; Elena & Lenski 1997), such a parameterization becomes troublesome at larger n because $w(n)$ could increase beyond the fitness of the reference sequence (for a positive coefficient in the quadratic term). This is avoided by the ansatz (Lenski *et al.* 1999)

$$w(n) = e^{-\alpha n^{\beta}}, \qquad (2.2)$$

where $\beta = 1$ means that there is no bias towards either form of epistatic interactions. $\beta > 1$ indicates that synergistic mutations prevail (mutations that are on average 'worth' more than one independent hit), while $\beta < 1$ reflects a bias towards antagonistic mutations (mutations that result on average in a 'damage' that is less than one independent mutation). (Note that in earlier works where a quadratic term was used, the distinction between the different types of directional epistasis depended on whether β was larger or smaller than zero rather than unity.) Since equation (2.2) depends only on two parameters, deviations from that form may arise when n grows large.

Naively, one might assume that the decay parameter α and the epistasis parameter β are independent. Instead, we shall see that environments with strong selection force a trade-off between α and β such that one can only be optimized at the expense of the other. The reasoning is as follows. In a strongly selective environment mutations can be classified as either neutral or lethal and $w(n)$ can be thought of as the fraction of neutral sequences in genetic space at mutational distance n. In particular, the neutrality ν of a sequence (the number of sequences at Hamming distance 1 with fitness 1) is related to the decay parameter by $\nu = l(D-1)e^{-\alpha}$, where l is the length of the sequence and D is the number of monomers. If all sequences in genetic space have the same ν, it follows that $\beta = 1$. A deviation from $\beta = 1$ implies that some sequences have more or fewer neutral neighbours than others, giving rise to a correlation between α and β. For a viable sequence with lower than average neutrality (higher than average α), there are comparatively fewer sequences close by than there are far away, such that this sequence will have a small β. Conversely, a sequence with a high neutrality (small α) will have comparatively more sequences close by and β will be larger. We can make this argument more formal with a simple 'conservation law', which only reflects the fact that the total number of neutral sequences in genetic space is constant. Since for polymers of fixed length l made from D monomers there are $\binom{l}{n}(D-1)^{n}$ possible n mutants, we must have

$$\sum_{n=1}^{l} w(n)\binom{l}{n}(D-1)^{n} = \mathcal{N}_{\nu}, \qquad (2.3)$$

where $\mathcal{N}_{\nu}$ is the total number of neutral mutants of this wild-type. Inserting $w(n)$ from equation (2.2) yields an implicit relation between α and β. Indeed, for two decay functions of two different reference sequences with different parameters α, the only way in which the sum can yield the quantity $\mathcal{N}_{\nu}$ (which is independent of the respective reference sequence) is that the two decay functions must have different parameters β as well. However, the implicit relation depends on the ansatz equation (2.2) being correct for all n, which is not necessarily the case. Alternatively, we may consider only sequences with up to d mutations, in which case we can write

$$\sum_{n=1}^{d} w(n)\binom{l}{n}(D-1)^{n} = \mathcal{N}_{\nu,d}, \qquad (2.4)$$

where $\mathcal{N}_{\nu,d}$ is the number of neutral sequences in a sphere of radius d around the reference sequence with $w(0) = 1$. $\mathcal{N}_{\nu,d}$ depends on the particular reference sequence chosen and, therefore, cannot be regarded as constant. However, for d not too small, we may replace it by its average $\langle \mathcal{N}_{\nu,d} \rangle$ over all viable reference sequences. If we take into account a sufficiently large region of genotype space, we should find approximately the same number of neutral mutants for each viable sequence, since, as d approaches l, the quantity $\mathcal{N}_{\nu,d}$ approaches $\mathcal{N}_{\nu}$, which in turn is independent of the reference sequence. Hence, equation (2.4) predicts a similar relation between α and β and the two different predictions approach each other as $d \to l$. Predictions based on equations (2.3) and (2.4) are used below for comparison with our empirical results.

Although the above argument strictly holds only under the assumption that mutations are either neutral or lethal, it is not unreasonable to assume that a similar (possibly weaker) correlation between α and β also exists in more general cases, where slightly deleterious or even advantageous mutations are possible. In that case, under the presence of epistasis, there will still be regions in genotype space in which the number of less-deleterious mutations is higher than average and other regions in which it is lower than average. The decay function $w(n)$ of a sequence from a region that is rich in non-lethal mutations would have a lower α, but would inevitably be more synergistic than the decay function of a sequence from a region poor in non-lethal mutations. Our results with digital organisms (see below) support this reasoning.

3. EXPERIMENTAL EVIDENCE

Accurate data for the decay parameter α and the epistasis parameter β for biological organisms are rare, which makes our hypothesis difficult to test. A few well-studied systems have emerged which are accessible *in silico*.

We studied RNA secondary structure prediction using the Vienna RNA package, version 1.3.1., with the default set-up (Hofacker *et al.* 1994). We calculated the decay of the average number of neutral folds as a function of the Hamming distance for 100 random RNA sequences of length $l = 76$. The parameters α and β were determined as follows. We obtained α exactly from the fraction of neutral mutants at Hamming distance one. In addition, we sampled the function $w(n)$ for Hamming distances up to $n = 8$ by calculating the structure of up to 10^{6} random
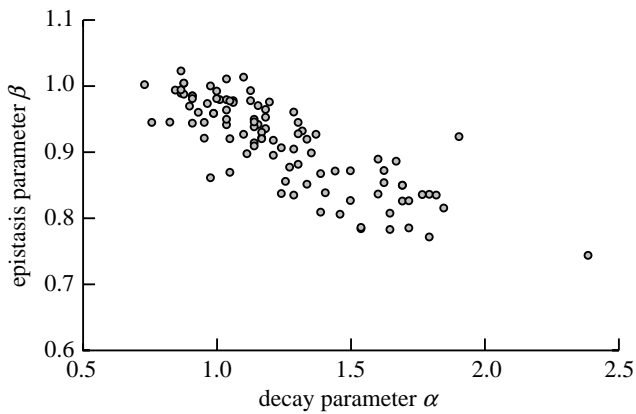
Figure 1. Epistasis parameter β versus the decay parameter α in random RNA sequences of length $l = 76$.

Figure 2. Epistasis parameter β versus the decay parameter α for sequences on the larger of the two neutral networks from Van Nimwegen *et al.* (1999). The solid line represents the prediction that follows from equation (2.3) and the dotted line represents the prediction that follows from equation (2.4), with $d = 8$.

neighbours of the required Hamming distance. The quantity β was then determined from a nonlinear fit of $-\alpha^{n\beta}$ to the logarithm of $w(n)$. A plot of β versus α (figure 1) shows a significant correlation, with a correlation coefficient of $r = -0.817$ ($p < 0.01$).

According to equations (2.3) and (2.4), we can predict the relationship between α and β if we compare the decay functions of sequences that are mutually neutral. For RNA folding, this means that we have to determine α and β for a set of sequences that fold into the same structure. We performed experiments with the RNA sequences of length $l = 18$ used by Van Nimwegen *et al.* (1999) (for these sequences we altered the default set-up by setting the free energies of dangling ends to zero). Two separate neutral networks (a neutral network is a network of neutral genotypes connected to each other by one-point mutations) consisting of 51 028 and 5169 sequences, respectively, were found by Van Nimwegen *et al.* (1999) for the particular case that all bonds are of the purine–pyrimidine type (G–C, G–U and A–U). Equation (2.3) predicts the correlation without a free parameter for each such set of neutral sequences, as long as the number of all neutral sequences $\mathcal{N}_\nu$ is known. In order to estimate $\mathcal{N}_\nu$, we generated 10^8 random sequences of length $l = 18$, of which 10 961 sequences folded correctly. From this, we estimated $\mathcal{N}_\nu = 7.5 \times 10^6$ neutral sequences out of the total 6.9×10^{10} sequences of length $l = 18$. Using this number, equation (2.3) predicts the solid line in figure 2, which describes the correlation well. The approach based on averaging over local neutral sequences around the reference sequence (equation 2.4) gives rise to the dotted line in figure 2 and shows that this too predicts the correlation fairly well (for this second approach we used $d = 8$ and from Monte Carlo simulations for 1000 reference sequences we obtained $\langle \mathcal{N}_{\nu,8} \rangle = 2.4 \times 10^5$).

As our second test case, we analysed the correlation between α and β in digital organisms (Adami 1998; Adami *et al.* 2000). Digital organisms are self-replicating computer programs that mutate and evolve. Their fitness is determined by the ratio of their central processing unit's (CPU's) speed and their gestation time. The latter is given by the number of instructions that have to be executed in order to generate a fully functional offspring. The CPU's speed increases when the digital organisms perform logical operations on numbers that they can
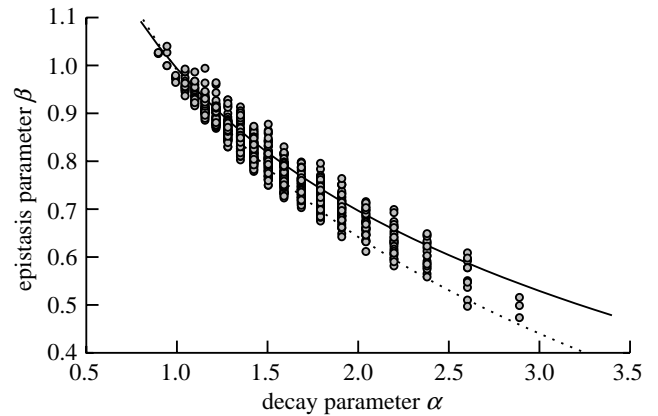
acquire from their environment. On the other hand, the gestation time increases if either the organisms employ less efficient mechansims of self-replication or if they accumulate instructions that are involved in the completion of the above-mentioned logical operations. The digital organisms with the highest fitness thus have a very efficient copy mechanism and perform a large number of logical operations with a comparatively small number of additional instructions. Lenski *et al.* (1999) measured the decay of the mean fitness as a function of the number of mutations accumulated in such digital organisms and obtained α and β from a fit of equation (2.2) to the measured decay functions. They studied 174 different genomes consisting of two groups of 87 genomes each. The first group of organisms evolved in 87 independent experiments in a complex environment, while the second group was obtained by transferring these organisms to an environment that favoured simple genomes and allowing the organisms to adapt to this more simple environment.

A statistical analysis revealed a significant correlation between the decay parameter α and the parameter of directional epistasis β for both the complex and the simple digital organisms (table 1). However, in addition to this correlation, we found a correlation between the α and both the genome length l and the log fitness for complex and simple organisms, as well as a correlation between β and l in the case of the complex organisms. Hence, for the complex organisms, we cannot rule out the possibility that the correlation between α and β merely reflects an underlying correlation of both quantities with length. In the case of the simple organisms, where we do not see a correlation between β and l, we can assume that the correlation between α and β is genuine. In order to provide further evidence, we examined a reduced dataset of all 48 simple organisms with a length between $l = 14$ and $l = 16$. These 48 organisms were also of comparable fitness. In that dataset, we found an even stronger correlation between α and β, while the correlation between either of the two quantities and length or fitness was insignificant (table 1). It was not possible to study a similar reduced dataset for the complex organisms

Table 1. The correlation r and p-value between decay parameter α, epistasis parameter β, length l and the logarithm of the fitness $\ln w$ in the data from Lenski *et al.* (1999).

(The 'complex' and the 'simple' datasets each consist of 87 digital organisms and the 'reduced' dataset consists of all 48 organisms of length between $l = 14$ and $l = 16$ taken from the 'simple' dataset.)

dataset	r	p
complex		
α and β	-0.599	< 0.01
α and l	-0.730	< 0.01
β and l	0.338	< 0.01
α and $\ln w$	-0.333	< 0.01
β and $\ln w$	0.112	> 0.05
simple		
α and β	-0.767	< 0.01
α and l	-0.502	< 0.01
β and l	0.058	> 0.05
α and $\ln w$	0.561	< 0.01
β and $\ln w$	-0.071	> 0.05
reduced		
α and β	-0.945	$\ll 0.01$
α and l	-0.101	> 0.05
β and l	-0.073	> 0.05
α and $\ln w$	-0.0289	> 0.05
β and $\ln w$	0.0466	> 0.05

Figure 3. Evolution of neutrality and epistasis as a function of time (in generations). (*a*) The change in β averaged over the population. The standard error of the mean does not exceed the size of the symbol for any data point. (*b*) The convergence of the population neutrality to the value predicted by the spectral radius of the connectivity matrix.

because the variations in length were too large (the length varied between $l = 20$ and $l = 314$ among the 87 genomes). Although the data from the digital organisms are potentially biased because our reference sequences were evolved rather than random as in the RNA case, we believe this bias to be insubstantial in at least the reduced dataset, which contains only organisms with very similar phenotypes. Hence, just as for our RNA data, the data from the digital organisms support our hypothesis of a genuine correlation between α and β.

4. ADAPTATION OF EPISTASIS THROUGH A CORRELATED RESPONSE

The correlation between neutrality and epistasis implies that, if one of them is subject to selective pressures, the other will be affected as well due to a correlated response. Van Nimwegen *et al.* (1999) showed that a population evolving on a neutral network reduces its genetic load by moving into the regions of high neutrality in sequence space. In particular, given a random population of molecules on such a network, evolution tends to increase the neutrality in the population, effectively pushing the population into the centre of the neutral network. Because of the correlation between neutrality and epistasis, we expect this dynamic to lead to a reduction in antagonistic epistatic effects. In order to verify this hypothesis, we carried out evolutionary experiments with the RNA sequences of length 18 from § 3.

We performed one flow reactor run for each of the two networks found by Van Nimwegen *et al.* (1999), starting with an initial population of 1000 sequences chosen at random from the respective network. We set the replication rates such that sequences folding into the target
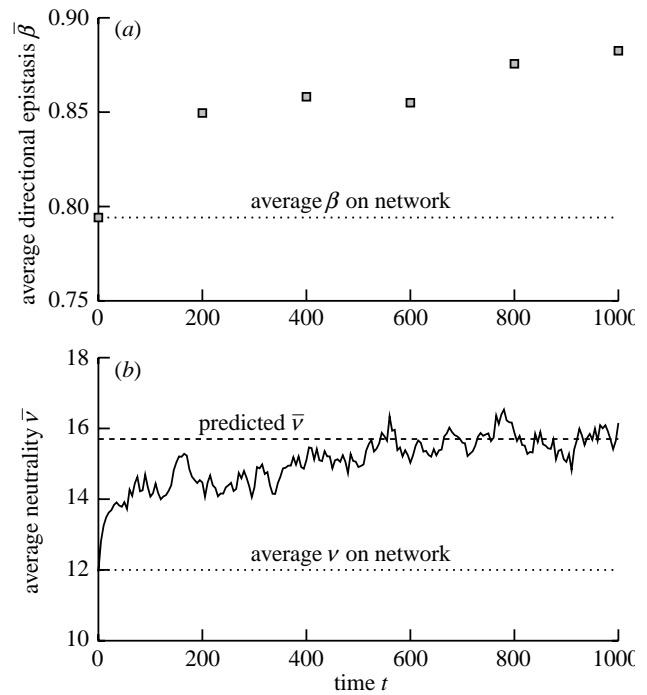
structure replicated on average once per unit time, while the replication rate of all other sequences was set to 10^{-6} per unit time. All sequences had a probability $\mu = 0.5$ of suffering one random point mutation per replication event. The possibility of several point mutations per replication event was eliminated in order to guarantee that the population could not leave the specified neutral network. The epistasis parameter β was determined for every sequence in the population every 200 generations, while the population neutrality was constantly monitored. The population neutrality $\overline{\nu}$ is the average neutrality of all sequences currently in the population. The results from the run on the larger of the two networks are presented in figure 3. The neutrality of the initial population coincides with the network neutrality (the average neutrality of all sequences on the network), which is to be expected for a random initial population. Over the course of evolution, the average population neutrality rose to the predicted equilibrium value (given by the spectral radius of the connectivity matrix) (see Van Nimwegen *et al.* 1999). As expected, the average epistasis parameter $\overline{\beta}$ increased significantly as well. The results on the second network were qualitatively identical, with β increasing from 0.78 to around 0.86. Thus, antagonistic epistasis is reduced during adaptation for reduced mutational load on a neutral network.

The above reasoning depends of course on the assumption that a population remains on a single neutral network. In a more realistic scenario, where peaks of different heights are present, the main effect of selection will be to increase the fitness rather than to increase α.

However, once a local optimum has been reached, we can expect the dynamic described above to take place, as long as there exists some neutrality at the local peak. If there is a correlation between the fitness and α or β, then we would additionally see a correlated response in α or β as the fitness is being maximized. Nevertheless, while the correlation between α and β reported here is a general result that follows from geometric constraints on the landscape, no such constraints exist between the fitness and α or β. The parameters α and β measure the amount and the distribution of neutral or nearly neutral sequences in the neighbourhood of a reference sequence. There is no reason why sequences with high fitness should generally be found in regions with particularly high or low neutrality or with a particular distribution of the neutral sequences. However, this is not to say that such a correlation cannot exist in special cases (see, for example, our data from the complex digital organisms in table 1 where α is correlated with fitness, but β is not).

5. CONCLUSIONS

Epistasis plays an important role in evolutionary theory, but remains empirically largely unexplored. Using secondary structure prediction of RNA sequences as well as digital organisms evolving *in silico*, we have demonstrated a correlation between two important parameters of realistic genetic fitness landscapes: the average deleterious effect of single mutations and the strength of directional epistasis. In conjunction with the results from Wagner *et al.* (1998) for mice and the two different theoretical explanations (a two-locus model in the case of Wagner *et al.* (1998) and a sequence space-based model here), we can expect this correlation to be a ubiquitous phenomenon that is present in many natural and artificial fitness landscapes.

This correlation, coupled with the selective pressure that forces random sequences in a neutral network to cluster in the dense areas of the network, leads to a reduction of strong antagonistic epistasis in a population. The nature of this result is purely geometric: as a population tries to reduce the average effect of single mutations, the effect of multiple mutations is inevitably worsened as long as there exist some inhomogeneities in the effect of single mutations across the genotype space. As a result of this geometric constraint, a member of an evolved population will on average have a higher β than a random sample of the fitness landscape would indicate.

It is well known that antagonistic epistasis favours the accumulation of deleterious mutations as well as the operation of Muller's ratchet. Since in such a situation sexual recombination (within a fixed environment) tends to worsen the loss of information, recombination is unlikely to evolve or be maintained. The mechanism described here may thus provide a path towards an environment more conducive to the evolution of recombination.

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