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**A Simple Model of Co-Evolutionary Dynamics Caused by Epistatic Selection**

Gerhard Schlosser\* and Günter P. Wagner#

\*Brain Research Institute  
University of Bremen, PO Box 330440  
28334 Bremen, Germany  
[gschloss@uni-bremen.de](mailto:gschloss@uni-bremen.de)

and

# Department of Ecology and Evolutionary Biology  
Yale University  
New Haven, CT 06520-8106, USA  
[gunter.wagner@yale.edu](mailto:gunter.wagner@yale.edu)

\*Corresponding author:

Dr. Gerhard Schlosser  
Brain Research Institute, AG Roth  
University of Bremen, FB 2  
P.O. Box 33 04 40  
28334 Bremen  
phone: 0421-218-3867  
fax: 0421-218-4549  
e-mail: [gschloss@uni-bremen.de](mailto:gschloss@uni-bremen.de)

**Abstract**

Epistasis is the dependency of the effect of a mutation on the genetic background in which it occurs. Epistasis has been widely documented and implicated in the evolution of species barriers and the evolution of genetic architecture. Here we propose a simple model to formalize the idea that epistasis can also lead to co-evolutionary patterns in molecular evolution of interacting genes. This model epistasis is represented by the influence of one gene substitution on the fitness rank of the resident allele at another locus. We assume that increasing or decreasing fitness rank occur equally likely. In simulations we show that this form of epistasis leads to co-evolution in the sense that the length of an adaptive walk between interacting loci is highly correlated. This effect is caused by episodes of elevated rate of evolution in both loci simultaneously. We find that the influence of epistasis on these measures of co-evolutionary dynamics is relatively robust to the details of the model. The main factor influencing the correlation in evolutionary rates is the probability that a substitution will have an epistatic effect, but the strength of epistasis or the asymmetry of the initial fitness ranks of the alleles have only a minor effect. We suggest that covariance in rates of evolution among loci could be used to detect epistasis among loci.

**Keywords**

Epistasis, coevolution, modularity, fitness landscapes, molecular evolution

## Introduction

Epistasis or gene interaction is the dependency of mutation effects on the genetic background. Epistasis for fitness can lead to situations in which a mutation is advantageous only in the context of certain other alleles at other loci, while it is deleterious in other situations. This form of dependency has been implicated in causing “rugged” fitness landscapes which can make genetic adaptation difficult (Kauffman and Levin, 1987; Whitlock *et al.*, 1995). These fitness landscapes have many local optima, i.e. locally optimal genotypes where it is impossible to reach higher fitness values without going through a “fitness valley.” Emphasis on “rugged” fitness landscapes forms the basis of Wright’s shifting balance theory of evolution (Wright, 1969). This intuition, however, as many ideas related to gene interaction, can be misleading. For one, fitness peaks are probably not generic features of epistatic fitness landscapes (Fontana and Schuster, 1998; Gillespie, 1984; Reidys *et al.*, 1997; Schuster *et al.*, 1994; Whitlock *et al.*, 1995). Instead models of gene interactions tend to have extensive ranges of equal fitness genotypes, also called neutral networks. Second, often epistatic fitness landscapes connect high fitness genotypes by shallow fitness ridges (Wagner *et al.*, 1994) allowing the evolution of alternative high fitness genotypes without crossing a deep fitness valley (Gavrilets, 2004). Both theories, the shifting balance model as well as the model of neutral networks, however, assume that evolution of epistatic genes requires the random fixation of either neutral or slightly deleterious mutations. But even this intuition does not generally apply, as has been shown recently for the epistatic interactions among amino acid residues in a protein.

DePristo and colleagues have shown that an adaptive mutation at the catalytic core of an enzyme regularly interacts with the fitness effects at other amino acid loci of the same protein (DePristo *et al.*, 2005). The explanation for this observation is that an adaptive mutation at the

catalytic core of an enzyme has consequences for the stability of the protein, leading to adaptive pressures at other amino acid residues to compensate for the loss of stability. Note that there is epistasis among different amino acid loci in that the optimal amino acid at a position depends on the amino acid in the catalytic core. Similarly the evolution of novel affinities in glucocorticoid receptors requires the interaction between mutations that change the affinity of the protein and those that accommodate the adaptive mutations in terms of stability (Ortlund *et al.*, 2007). The evolutionary dynamic, driven by environmental factors and epistatic interactions, only includes adaptive changes and has no need for random drift either along neutral networks or through fitness valleys. To our knowledge this form of epistatic dynamics has not been modeled before, as most of the theoretical work has focused on either the population genetic details of the shifting balance model or the structural features of neutral networks (Gavrilets, 2004).

In this paper we present a simple model in which two loci undergo adaptive evolution by simple mutational hill climbing, but also interact by affecting each others fitness rank among alternative alleles. This model was originally motivated by the idea that genomes consist of modular networks of interacting genes (Schlosser, 2002, 2004). Given these modules of interacting genes it was hypothesized that the members of a gene network module should form a set of genes that tends to co-evolve because of their epistatic interactions (Schlosser, 2002). Some adaptive substitutions at one locus can induce selection pressures at other loci in the same network causing them to undergo adaptive evolution in turn, similar to the amino acids in an enzyme, as exemplified in the work of DePristo *et al.*, (2005). This idea raises the possibility that functional modules may be detectable through co-evolutionary dynamics revealed by the comparison of genome sequences, similar to the detection of physically interacting residues in RNA and proteins (Fariselli *et al.*, 2001; Gobel *et al.*, 1994; Pang *et al.*, 2005; Pazos *et al.*, 1997; Taylor and Hatrick, 1994).

Here we analyze the dynamical properties of this model asking how likely epistatic interactions leave a statistical signature in the pattern of gene substitutions. We show that epistasis leads to co-evolution among the loci and that the outcome is relatively robust against details of the model. We further show that the adaptive dynamics of non-epistatic and epistatic gene pairs can be distinguished based on their correlated pattern of adaptive substitutions with relatively high power if epistasis is prevalent.

## The Model

We consider a haploid genotype with two loci. Each locus has  $n$  possible allelic states. Alleles at a locus are represented by an integer that gives their fitness rank  $r$  in a given genetic background among the set of possible alleles at the locus. With  $n$  possible allelic states the rank  $r=n$  is the optimal allele in a genetic background, and  $r=1$  the worst. The model represents the direct effects of mutations on the fitness rank of an allele as well as the epistatic effects on the other locus (Fig. 1).

We assume that in each generation there may be maximally a single mutation with a fixed predetermined probability  $m$  (mutation rate) and that this mutation may be in either locus A or B with equal probability. Hence we are simulating the limiting case of low overall mutation rate, in which the rate of evolution is determined by the availability of advantageous mutations rather than by selection on standing variation.

Mutations change the fitness rank according to a given distribution. If the fitness rank of the mutant allele is higher than that of the old allele, the new allele replaces the old one in the genome. That means we simulate instantaneous fixation of the new allele, assuming that the time

to fixation is short compared to the waiting time for the next mutation. If the mutant fitness is less than the original one, the mutation is discarded.

The fitness rank of a new mutation can be determined by any probability distribution over the set of possible allelic states. Here we assume, for simplicity, that the probability of higher or lower fitness follows a uniform distribution. In this way the probability of an adaptive mutation (one that ranks higher in fitness than the original allele) decreases with the fitness rank of the allele and the rate of evolution slows down proportionally. The probability of an adaptive mutation is  $P_a = \frac{n - r}{n - 1}$ , and for further reference we call  $n - r = n_2$ , i.e. the number of possible alleles with higher fitness rank than the given allele.

Epistatic effects are represented in this model as influence of an allele substitution at one locus on the fitness rank of the current allele at an interacting locus according to some probability distribution. The prevalence of epistatic effects between the two loci is defined via a parameter  $E$ , which determines the probability that mutations in one locus epistatically affect the fitness rank order at the other locus. The strength of epistatic effects, on the other hand is determined by the shape of the probability distribution that determines the change in fitness rank due to epistasis.

In order to model the epistatic effects on the second locus after a substitution at the first locus we chose a distribution of epistatic effects that has the following characteristics. The epistatic effect will equally likely increase the fitness rank as well as decreasing it. This assumption is motivated by the empirical finding that epistasis among fitness effects has no preferred direction in *E. coli* (Elena and Lenski, 1997, 2001). Because there is a finite number of ranks in our models, this assumption has to be modified at the boundaries, i.e. when either maximal or minimal ranks are reached. At these boundaries we use the same distribution as if the rank of the allele were the second highest or second lowest allele, respectively and was equally



likely to increase or decrease (see below). In order to have a distribution which respects the finite size of the range of possible allelic states we chose a distribution which is based on a Beta distribution. The Beta distribution is defined on the unit interval between zero and one, and can thus be mapped onto any finite interval and discretized to yield defined probabilities for each allelic state.

Briefly, we use a Beta distribution where the random variable  $x$  is the relative rank of the allele after epistasis has taken effect:  $x | \frac{r' - 1}{n - 1}$ , with  $r$  being the rank before the epistatic effect and  $r'$  after the epistatic effect. Note that  $x \in [0, 1]$ , as assumed for the support of the Beta distribution. To determine the epistatic effect on an allele with a certain initial fitness rank  $r$  we choose a distribution with the median  $M$  equal to the relative rank of the original fitness rank:

$M | \frac{r - 1}{n - 1}$ . This ensures that the epistatic effect will equally likely increase the fitness rank as well as decreasing it (Fig. 2).

To define the parameters of the epistatic effect distribution we use the following parametrization:

$$p(x) = \frac{x^{a-1} (1-x)^{k-a-1}}{B(a, k-a)}$$

where  $B(a, k-a)$  is the Beta function. The variance of the distribution is largely determined by the parameter  $k$ ,

$$\text{Var}(x) = \frac{a/k - a^2}{k^3 - 2k}$$

such that increasing  $k$  decreases the variance of  $x$ . The expected value of  $x$  is

$$E/x = \frac{a}{k}$$

Hence we have two parameters,  $k$  and  $a$ . We choose to fix  $k$  as a way to determine the shape of the distribution (strength of the epistatic effect) of allelic effects for a given simulation. The distribution also depends on the fitness rank of the current allele, because  $r$  determines the median of the distribution (see above). Hence we need to determine the parameter  $a$  for a given fitness rank by solving the following integral equation

$$0.5 = \frac{1}{B(a, k+1)} \int_0^M x^{a+k} / \Gamma(a+k) dx$$

for  $a$ , with a fixed predetermined  $k$  and  $M = \frac{r-1}{n-1}$ . By definition  $M$  is the median of the distribution, equal to the relative rank of the allele before the epistatic effect. To our knowledge there is no analytical solution to this integral equation and we thus numerically solved this equation in our simulation to determine the distribution of epistatic effects.

The method to determine the distribution of epistatic effects as outlined above works as long as the current rank of the allele is neither maximal,  $r=n$  or minimal,  $r=1$ . In these cases the allele can either not increase or decrease in rank, respectively. As explained above, in these cases we determine the distribution of epistatic effects under the assumption that the allele occupied the

second highest or second lowest rank, respectively and was equally likely to increase or decrease.

In other terms, we either set  $M = \frac{1}{n-4}$  if  $r=n$ , or if  $r=0$  we set  $M = \frac{1}{n-4}$ .

Since this model is a highly stylized representation of evolutionary and genetic dynamics we summarize here the assumptions that are implicit in this model structure and discuss ways to make the model more realistic.

#### *## Strong Selection Weak Mutation:*

- At any one time only one of the two loci is polymorphic
- Selection happens much faster than the waiting time for mutations
- Fixation probabilities of advantageous mutations do not vary among alleles.

These assumptions allow us to ignore the details of population dynamics and make the evolutionary process a case of simple hill climbing. The last assumption can be changed by replacing the fitness ranks with actual fitness values and calculating the fixation probabilities from the fitness differences.

#### *## Rank Epistasis*

- We only consider cases where epistasis changes the fitness rank of an allele.  
This is a generalization of the more commonly used concept of sign epistasis in the two-allele case, where the sign of a fitness effect is reversed due to epistasis.
- The fitness rank of a mutant follows a uniform distribution over all alternative alleles at a locus. This assumption is the simplest which leads to a decreasing rate of advantageous mutations as the fitness rank of mutations increases. This assumption is easily replaced with the more realistic models

of gene substitution and fitness change based on extreme value theory (Orr, 2002).

- We interpret the fitness rank effect of a mutation as representing the fitness of the haplotype containing both loci. This implies that the epistatic change in fitness rank of the allele at the second locus does not influence the fixation probability.

An adaptive mutation at one locus can lead to fitness rank decreases at the second locus in two ways. It can release a constraint on the adaptation of the second locus, which means that alleles that become advantageous after the first mutation lead to a higher fitness than was possible before the first mutation. In this case the epistatic effect would not be associated with a deleterious pleiotropic effect and thus would not affect the selection coefficient of the mutation. Alternatively we can assume that the fitness rank at the second locus is influenced by deleterious pleiotropic effects of the first mutation, as it seems to be the case of the protein adaptation model by DePristo et al., (2005). In this case we have to assume that the fitness consequences of the pleiotropic effects are less than the direct fitness gain due to the first mutation, e.g. catalytic activity versus protein stability. To relax this assumption would also require abandoning fitness ranks and replace them with explicit real valued fitness values.

#### *## Sparse genotype space covering*

- We assume that in any simulated evolutionary process the number of realized genotypes is a tiny fraction of all possible genotypes.

This assumption is necessary to ensure topological consistency of the fitness landscape assumed in this model. Since genotype fitness values are not explicitly stated in the model, but are rather represented by a random field, as in the  $N_k$  model (Kauffman and Levin, 1987) it is certain that our model violates topological constraints of possible genotype fitness mappings. The

most important is the fact that epistatic effects are symmetrical, i.e. the epistatic effect of a substitution at one locus on the other locus is the same as the epistatic effect of the mutation at the second locus on the first (Wagner et al., 1998). This symmetry is a statement about pairs of alleles at two loci, but has also implications for our model. One of which is that it is impossible that one locus only has effects on the other but not vice versa. Some asymmetries in the probability of interaction are thinkable but imply that the two sets of alleles are also unequal. We decided to not pursue this possibility. We thus assume that the probability that a mutation at one locus has an epistatic effect on the second is equal to the inverse epistatic effect.

To analyze the effect of epistatic interactions between two loci on their evolutionary dynamics, we simulated evolutionary changes in the fitness ranks of two loci under the assumptions of the model described above for different parameters of epistatic prevalence  $E$  and epistatic strength  $k$ . The mutation rate was always 0.2 and the number of fitness ranks for each locus was 1000. Unless otherwise noted, the initial rank of each locus at the beginning of simulations was 500. However, in order to analyze whether differences in the initial ranks of the two loci affect the co-evolutionary dynamics of the process, we also explored cases, where one locus started at rank 1000 (i.e. maximal rank), while the other started at rank 500. For each set of parameter values, we analyzed 1000 replicate runs. Each run proceeded through 100,000 iterations, when no further evolution occurred in the overwhelming majority of runs because the highest rank was reached for both loci even under assumptions of strong and prevalent epistasis. The model was realized in Mathematica 5.0 and is available upon request from the corresponding author.

## Results

Figures 3 and 4 show a number of typical runs depicting the evolution of fitness ranks of the alleles at the two loci. Figure 3 presents examples without any epistatic interactions and Figure 4 presents examples with prevalent epistasis. The patterns of rank evolution are evidently much different. In the absence of epistasis (Fig. 3), decreases of rank in any of the loci are impossible because only mutations, which increase fitness rank are fixed. In each of the three cases illustrated (Fig. 3A-C), each locus initially rapidly climbs up the ranks but the time interval between substitutions markedly increases as it approaches maximal rank because the probability for an advantageous mutation decreases. As expected from our assumption of independence, the time course of substitutions in the two loci is completely unrelated to each other.

In the presence of epistasis (Fig. 4), a substitution in one locus may either raise or lower the fitness rank of the allele at the second locus due to positive or negative epistasis, respectively, allowing for the possibility of rank decreases. Three examples are illustrated. In the first example (Fig. 4A), negative epistatic effects in both loci lead to brief interruptions of the initial climb in ranks, e.g. at iteration  $t=304$ , where a substitution in locus A from rank 877 to 985 epistatically drives down the rank of B from 942 to 457. However, positive epistatic effects also occur and in this case they contribute to a more rapid achievement of near-maximal ranks in both loci than in typical cases without epistasis. This is the case with a substitution at  $t=648$  in locus B from rank 929 to 997, which epistatically brings A from 998 to 1000. Two further substitutions in B without negative epistatic effects on gene A eventually bring both loci to maximal rank but due to the very low probability for occurrence of advantageous mutations close to maximal rank this requires more than 7500 additional iterations. In fact in this model if a locus is near its maximal fitness rank an epistatic effect is more likely to increase fitness than a mutation, because the

probability of an adaptive mutation decreases with fitness rank, while the probability of increased fitness rank due to epistasis remains at 0.5.

The second and third examples in Figure 4 are representative for many cases, where after an initial climb to near-maximal ranks in both loci long periods of stasis are interrupted by brief episodes of co-evolutionary bursts. In the second example (Fig. 4B), a substitution in locus B (from rank 994 to 997) epistatically brings gene A from rank 866 to 992 at  $t=343$  followed by a relatively quiescent period in both loci until  $t=8597$ , when a substitution in gene A (from rank 999 to 1000) initiates the first co-evolutionary burst by bringing B epistatically down from rank 1000 to 920. This low rank in gene B enhances the probability for advantageous mutations in gene B resulting in a more rapid succession of substitutions in B and a correspondingly elevated frequency of negative epistatic effects on gene A. A substitution in gene B from rank 977 to 980 at  $t=9092$  pulls down A from rank 999 to 656 inducing the same changes of probability in the other locus. A brief episode of rapid substitutions in both loci (24 substitutions in 2015 iterations) with positive and negative epistatic effects follows until the burst ends at  $t=10612$  with a substitution in A from rank 982 to 999, which raises the rank of B from 905 to 1000 by positive epistasis. This initiates another long period of stasis until a second co-evolutionary burst at  $t=29699$  after 7 substitutions in 557 iterations culminates in the convergence of both loci to maximal ranks at  $t=30256$ . The third example (Fig. 4C) shows another case with an initial climb followed by stasis interrupted by two co-evolutionary bursts. The first more protracted burst is initiated between  $t=4500$  and  $t=12795$  by a substitution in gene B (from rank 988 to 994), which epistatically pulls down the rank of A from 1000 to 584. A second co-evolutionary burst occurs between  $t=20447$  and  $t=22730$  after a substitution in gene A from rank 998 to 999 epistatically decreases the rank of B from 1000 to 914.

Comparing the cases with (Fig. 4A-C) and without (Fig. 3A-C) epistatic effects reveals a much larger disparity of evolutionary trajectories between different runs in the presence of epistasis. Nevertheless, the evolutionary trajectories of the A and B locus in a single run are strikingly similar in the case of epistasis indicating a high tendency of both loci to co-evolve while showing no obvious correlation in the absence of epistasis. Despite the differences between runs in case of epistasis, co-evolutionary bursts are typically initiated by a substitution with negative epistatic effect in situations, where both loci have near-maximal rank. Positive epistasis, on the other hand often plays a role during termination of such co-evolutionary episodes.

We next compared the distribution of substitutions per locus until convergence under assumptions of prevalent and strong epistasis ( $E=1$ ,  $k=2.1$ ) with the distribution of substitutions under assumption of no epistasis ( $E=0$ ) (Table 1, Fig. 5). The mean and median of the distribution are significantly higher in runs with prevalent epistasis than in runs without epistasis (15.2 and 12 versus 6.7 and 7, respectively), the standard deviation is much higher (11.3 versus 2.2), and the distribution is much more skewed towards higher substitution numbers. The differences in mean and median reflect the delay of convergence in the presence of epistatic interactions due to the possibility of negative epistatic interactions and their promotion of bursts of rapidly alternating substitutions at both loci. The higher standard deviation and skew in cases with epistatic interactions reflects the fact that the number and duration of such co-evolutionary bursts is highly sensitive to the stochastic influences of both actual mutational and epistatic effects. While the total number of substitutions until convergence are completely uncorrelated between the two loci A and B of a single run in the absence of epistasis (Fig. 6A), they are strongly positively correlated ( $\psi=0.868$ ) in cases with strong and prevalent epistasis (Fig. 6B). This correlation reflects the fact that negative epistatic effects of a substitution in one locus on the other locus increase the probability of another substitution at the other locus and, consequently, also increase



the probability for substitutions with negative epistatic effects on the first locus, leading to a correlation of substitution frequencies by positive feedback.

We also compared the distribution of total duration of runs until convergence and of total substitution rate (number of substitutions until convergence/duration of run) under assumptions of prevalent and strong epistasis ( $E=1$ ,  $k=2.1$ ) with replicate runs under assumption of no epistasis ( $E=0$ ) (Table 2). While convergence on maximal ranks occurs on average slightly later in runs with epistasis than in runs without epistasis (19416 vs. 15262 time steps, respectively), this difference is at most marginally significant (Mann-Whitney test:  $P=0.0596$ ). The total substitution rate, however, is significantly higher (Mann-Whitney test:  $P=0.000$ ) under assumption of epistasis than without epistasis (16.73 vs. 7.98 substitutions/5000 time steps, respectively) (Table 3, Fig. 7A).

To better understand how substitution rates are distributed under epistatic and non-epistatic conditions, we first subdivided each run into relatively long time windows of 5000 iterations and counted the total number of substitutions in each time window, which allowed us to determine the rate of substitution for each time window (Table 4, Fig. 7B). The last time window was discarded, whenever it was shorter than 5000 time steps. This implies that runs, which were shorter than 5000 time steps did not enter into the calculations and a number of substitutions at the end of each run had to be discarded (consequently, the average values of rates calculated for time windows differ notably from the total substitution rates).

Under both epistatic and non-epistatic conditions the substitution rates in time windows of 5000 steps show a first peak at 0-2 substitutions/5000 time steps reflecting the long periods of stasis when loci have reached near-maximal ranks. Under non-epistatic conditions there is a second peak centered around 10-16 substitutions/5000 time steps, which is due to the initial climb to near-maximal ranks as confirmed by the disappearance of this peak, when the initial two time

windows are omitted from the analysis (not shown). Under epistatic conditions, there is a more even distribution of rates: there are fewer time windows that have either very few or around 10-16 substitutions/5000 time steps than under non-epistatic conditions, but more time windows that have rates in between or larger than 16 substitutions/1000 time steps. This is probably due to the fact that on the one hand positive epistatic effects may decrease the rate of substitutions in the initial climb to near-maximal rank, while on the other hand negative epistatic effects may increase rates by increasing the probability for additional substitutions, when loci occupy near-maximal ranks (Fig. 7B). This may result either in merely brief interruptions of convergence or may initiate co-evolutionary bursts of highly variable duration and density of substitutions, the variability being due to the stochasticity of actual mutational and epistatic effects. Taken together this suggests that while epistasis strongly affects substitution rates, positive and negative effects on substitution rates may partly compensate for each other.

One of the most striking differences between simulations with and without epistasis is the occurrence of bursts of rapidly alternating substitutions in both loci (co-evolutionary bursts) under the former condition. To quantify the degree to which substitutions in the two loci are temporally clustered, we analyzed the distribution of waiting times between those substitutions at one locus that were followed by a substitution at the other locus (alternating substitutions). Average waiting times are significantly (Mann-Whitney test:  $P=0.000$ ) shorter under epistatic conditions than under non-epistatic conditions (Table 5).

We next tested, whether the occurrence of co-evolutionary bursts of substitutions in both loci under epistatic conditions is reflected in correlations between the rates of substitution in locus A and B in different time windows of the same run. We, therefore, subdivided each run into shorter time windows of 500 iterations and determined the substitution rate of each locus for each time window. The last time window was discarded, whenever it was shorter than 500 time steps.

We then determined the correlation between substitution rates in locus A and B for each run. Because correlations can only be meaningfully calculated if a sufficient number of data points are available, only runs that covered at least 20 time windows were included in the analysis. We also excluded the first time window of each run from the analysis because our simulations started at rank 500 of 1000 and this allowed rapid initial accumulation of substitutions in both loci for both the epistatic and non-epistatic case, which misleadingly suggest relatively high correlations for the non-epistatic case. The distribution of correlations between substitution rates in A and B under conditions of strong and prevalent epistasis and under non-epistatic conditions are compared in Figure 8A. The average correlation of substitution rates is much higher under epistatic conditions (see also Table 6). However, there is some overlap of the distributions under epistatic and non-epistatic conditions. The cumulative density functions reveal that while 95 % of the non-epistatic runs have correlations below 0.639, this is also true for 60% of runs under epistatic conditions indicating that around 40% of the runs with co-evolution of both loci due to epistasis can be detected allowing for a 5% type I error. This means that it would be difficult to detect epistatic interaction in real data sets based on the correlation of number of substitutions. However, the overlap of the distributions decreases (Table 6, Fig. 8B) and the power to detect co-evolution due to epistasis increases correspondingly, if more time windows at the beginning of each run are excluded further reducing artifactual correlations due to the initial conditions (high substitution rates in both loci due to their initially low rank assignments). After exclusion of the first four time windows, for example, already around 80% of runs with co-evolving loci due to epistasis can be detected with a 5% type I error (Fig. 8B).

In order to analyze the effect of the strength of epistatic interactions, we next compared the total number and rates of substitutions per locus until convergence to maximal ranks in 1000 replicate runs for conditions with prevalent epistasis ( $E=1$ ), but with varying strength of epistatic

effects. We varied the strength of epistatic effects by changing the shape parameter  $k$  of the Beta distribution used to determine epistatic changes in fitness rank. Increasing  $k$  decreases the variance of epistatic effects (Fig. 2A) and thus the average epistatic effect – the average change in rank after epistasis (Fig. 2B). Surprisingly, however, decreasing epistatic strength from  $k=2.1$  to  $k=6.1$  had only a minor effect on the mean, median and standard deviation of the distribution of substitutions until convergence (Table 1). Moreover, the correlations between total number of substitutions until convergence at the two loci of a single run are uniformly high across the range of  $k$  tested (ranging from  $\psi=0.840$  at  $k=6.1$  to  $\psi=0.868$  at  $k=2.1$ ) (Table 1, Fig. 9A). Similarly, the average correlations between the substitution rates of both loci in different time windows of same run remain relatively constant for different  $k$  (ranging from  $\psi=0.470$  at  $k=6.1$  to  $\psi=0.574$  at  $k=2.1$ ) (Table 6, Fig. 9B). This indicates that changing the strength of epistatic interactions within the range of  $k$  tested has only a minor effect on the co-evolutionary dynamics of the two loci and that the co-evolutionary dynamics is robust to details in the distribution of epistatic effects.

Varying the prevalence or probability of epistasis  $E$ , in contrast, strongly affects the probability of the loci to co-evolve. Comparing the total numbers of substitutions per locus until convergence to maximal ranks in 1000 replicate runs with  $E$  ranging from 0 to 1 we find a strong effect on the mean, median and standard deviation of the number of substitutions, which are gradually increasing with increasing values of  $E$  (Table 1). Similarly, the correlations between total number of substitutions until convergence at the two loci of a single run increase with increasing  $E$  (from  $\psi=0.004$  for  $E=0$  to  $\psi=0.868$  for  $E=1$ ) (Table 1, Fig. 9C). Likewise, the average correlation between substitution rates of both loci in different time windows of same run increase with increasing  $E$  (from  $\psi=0.168$  for  $E=0$  to  $\psi=0.574$  for  $E=1$ ) (Table 6, Fig. 9D).

Finally, we investigated how sensitive the co-evolutionary dynamics of our model is to variations in the initial rank assignments for both loci. While all simulations reported so far

started with symmetrical initial conditions, in which both loci occupied an intermediate rank of 500, we now analyze cases with highly asymmetrical initial conditions, in which locus A starts at its maximal rank 1000, while locus B starts from an intermediate rank of 500 (Table 7, Fig. 10). The distributions of total number of substitutions until convergence in both loci as well as the correlations between the total number of substitutions at the two loci of a single run are largely unaffected by this alteration in initial conditions (Fig. 10 A, B). However, the average number of substitutions under asymmetric conditions is lower for locus A than for locus B. With declining prevalence of epistasis  $E$ , the distribution of substitutions in locus A deviates increasingly from locus B and shifts towards zero because the probabilities for its escape from maximal rank due to epistasis decrease (Fig. 10 C,D). As a corollary of this shift of the distribution towards zero with declining  $E$ , the correlation between total number of substitutions appears to be increasingly elevated under asymmetric compared to symmetric initial conditions (Fig. 10B). The elevated correlations under asymmetric conditions should, therefore, not be taken as an indicator of increasing co-evolution probabilities between the two loci.

## Discussion

Different species co-evolve, when the fitness, i.e. the propensity for survival and reproduction, of at least one of the species depends on particular properties or behaviors of the other (as in the case of mimicry or of flower-pollinator interactions). However, co-evolution should even be more important and prevalent between different components of the same organism. Organisms are complex systems, which require proper integration and cooperation among many components to perform their functions. Because the fitness effect of any heritable

variation of a component (e.g. a mutation at a particular gene locus) depends on whether this functional integrity is preserved, it will depend on those other components (loci) with which it closely cooperates in performing a certain function (Schlosser 2002). Fitness interdependence between the two loci will be reflected in a certain probability of epistatic effects. That is, the fitness effect of a substitution in locus A may differ depending on which allele is present at locus B.

Co-evolution has been inferred from correlations in the rate of evolution, but only recently co-evolution has been formally demonstrated experimentally (for a review see Fraser, 2006). For instance the genes *fem-1* and *tra-2* are transcription factor genes in the sex determination pathway of nematodes. The protein products of these genes directly interact and their amino acid sequences evolve quickly as shown by a comparison of three *Caenorhabditis* species. In all three species these genes are essential for sex determination but the protein-protein interaction is only possible among proteins from the same species (Haag et al., 2002). Similarly co-evolution has been demonstrated in two yeast species among the cyclin Pcl5 and its substrate, the transcription factor Gcn4 (Gildor et al., 2005). While it is still not clear what drives the rapid co-evolution among those genes, it seems unlikely that they depend on random drift. Finally it has been demonstrated that genes in functional modules of yeast in fact do have more similar rates of evolution than genes from different modules (Chen and Dokholyan, 2006). Hence it seems that there is a need to understand in greater detail the co-evolutionary dynamics among epistatically interacting genes.

The model we present here analyzes how fitness interdependence between two loci due to fitness epistasis affects their evolutionary dynamics. Our focus in this model is on long-scale evolutionary trajectories and we neglect population-dynamics assuming instead instant selective fixation of any advantageous mutation. This is similar to Gillespie's SSWM model, where SSWM stands for Strong Selection Weak Mutation (Gillespie, 1991). Importantly, our model

assumes that only one of the two loci is polymorphic at any given time. Thus, in contrast to many previous models of epistasis (Goodnight, 1988, 1995; Hansen and Wagner, 2001; Kauffman and Levin, 1987; Stadler, 1996; Stadler *et al.*, 2000), our model does not consider epistasis among several simultaneously polymorphic loci. Instead, it investigates how evolution at one locus depends on the outcome of previous substitutions at another locus. Specifically we model epistasis as the effect of one substitution on the fitness rank of alleles at another locus.

The model is a random field model, i.e. instead of defining the fitness values of each genotype directly it only makes assumptions about the statistical distribution of effects. It is thus in spirit similar to the  $N_k$  model of Kauffman and Levin (1987). The main difference of our approach is that we use a random model to determine epistatic effects rather than genotypic fitness directly. The advantage is that the statistical properties of the epistatic interactions are very clear and explicitly stated, but this comes at a price. The disadvantage is that this random model is not guaranteed to respect the topological constraints of fitness landscapes, the main one is the symmetry of  $A \times A$  epistatic effects, i.e. that the epistatic effect of locus A on B is the same as the epistatic effect of B on A (Wagner *et al.*, 1998). This problem only plays a role, however, if the number of genotypes sampled in any simulation approaches the size of the genotype space. That is to say, that any small sample of epistatic effects can always be embedded in a larger fitness landscape without violating the symmetry conditions of fitness landscapes.

Even though this model uses a statistical approach in describing epistatic interactions it is a model based on “physiological” epistasis (*sensu* Cheverud and Routman, 1995), as it determines the fitness values of genotypes. In this model epistasis thus does not refer to epistatic variance components, or what has been called “statistical epistasis”. In fact according to the assumptions of the model there is no statistical epistasis at all, since we assume that only one locus is polymorphic at any time and the effect of this locus is by definition additive. That is so because

there can only be an epistatic variance component if at least two loci are polymorphic at the same time.

Another question important to the interpretation of this model is whether the parameters of the model have a clear biological and operational meaning. Often epistasis is introduced into mathematical models as un-interpreted nonlinearities (e.g. in Wagner et al., 1994, Gavrillets and de Jong, 1993 and others), an approach that has been criticized (Wagner et al., 1998; Hansen and Wagner, 2001). In this model epistasis is described by two factors: the probability of an interaction  $E$  and the distribution of fitness rank changes if an epistatic effect occurs. Both parameters have a straightforward operational interpretation, even though it is difficult to estimate them in reality. For instance, in the case of amino acids in a protein the probability of interaction  $E$  can be estimated as the fraction of amino acids substitutions at one position that lead to a change in fitness rank at another amino acid position. This estimation assumes that all amino acids have the same probability to replace any other amino acid, but that can be modified by taking into account the structure of the genetic code and the nucleotide mutation rates. In general the parameter  $E$  may depend on the current amino acid at a position, however. We found that the probability of interaction has a major influence on the behavior of the model, in particular on the correlation between the number of substitutions at the two loci.

In our model the epistatic effect on locus B of a mutation at locus A is modeled by a Beta distribution on the relative fitness rank of alleles at locus B. Of course there is no guarantee that in reality this distribution will resemble a Beta distribution. This choice was dictated by mathematical convenience, as the Beta distribution is the generic family of probability density functions on the unit interval. So the real question is whether describing an epistatic effect with a probability distribution has an operational interpretation. Let us focus on a specific allele  $b$  at locus B and create a series of mutations at locus A. For each mutation we determine the change in



fitness rank at of  $b$  due to epistasis with the change on locus A. Given the original fitness rank of  $b$  the fitness rank changes will define a frequency distribution. It is this frequency distribution that is represented by a Beta distribution in our model. This is in principle an operational definition of our parameters but measuring these parameters clearly requires a large amount of data. Only with very efficient methods of site directed mutagenesis and fitness evaluation this data can be produced, but recent advances in high throughput technology suggest that data of sufficient quality to estimate these parameters might be obtainable in the near future. In our simulations we found that the shape of the Beta distribution does not affect the model dynamics much and we thus infer that the model is robust against assumptions about the exact distribution of epistatic effects.

Comparison, in our model, between evolutionary trajectories of epistatically interacting loci with those of non-epistatic loci suggests that epistasis between two loci should lead to co-evolution, as recently demonstrated in yeast interaction networks (Chen and Dokholyan, 2006). As measures of co-evolution we determined the correlation of total number of substitutions until reaching maximal rank between two loci of the same run as well as the average correlation between substitution rates of both loci in different time windows of the same run. While substitution numbers between the two loci are completely uncorrelated in the absence of epistasis, they are strongly correlated in the presence of epistasis (Fig. 6). Moreover, substitution rates of both loci in different time windows of the same run are correlated much more frequently in the presence of epistasis, indicating that the temporal pattern of substitutions in two epistatically interacting loci tends to be similar (Fig. 8).

One of the most distinctive effects of epistasis in our model is that it does not lead to a temporally homogeneous elevation of substitution rates in both loci but rather promotes co-evolutionary bursts – periods of elevated substitution rates in both loci which alternate with long

periods with few or no substitutions (Fig. 4). These co-evolutionary bursts are probably due to positive feedback by negative epistatic effects: by increasing probabilities for substitution at one locus they increase the probabilities that the rank of the other locus is decreased by negative epistatic effects raising the probabilities for substitution at that locus as well.

The latter feature of our model contrasts with the prevalent model of correlated evolution, in which it is assumed that the first step is a slightly deleterious mutation that gets fixed by random drift and then is compensated by selection on a compensatory mutation (Stephan, 1996). In this model one slowly fixing mutation is followed by a selected substitution. In contrast, in our model all the substitutions are adaptive and bursts of substitutions are caused by the epistatic effects of an adaptive change leading to what has been called epistatic selection.

These co-evolutionary bursts reflect the biologically plausible situation, where gene substitutions in the wake of an environmental change at one locus may actually decrease the level of co-adaptation of both loci and thus induce selection for compensatory change at the other locus. For example, a mutation of a receptor may be advantageous (i.e. increase haplotype fitness) because it increases its ability to faithfully activate an intracellular second messenger (e.g. by increasing its affinity to the latter) even though as a side effect (e.g. due to allosteric effects) it slightly compromises the receptors ligand-binding capacity. As a consequence, substitutions in the ligand would now be favored that restore high-affinity ligand binding to the modified receptor (e.g. because they fit better in its allosterically modified ligand-binding site) even though they may have been unfavorable prior to the receptor modification.

Correlated evolution of sequences has been documented quite often and has been used to detect physically interacting nucleotide and amino acid residues (Fariselli *et al.*, 2001; Gobel *et al.*, 1994; Pang *et al.*, 2005; Pazos *et al.*, 1997; Taylor and Hatrick, 1994). Correlated amino acid substitutions, however, are not limited to directly interacting amino acid residues, as recently

been shown by Buck and Atchley (2005) in the case of Serpin proteins. In addition there is ample evidence for the occurrence of sign epistasis where an allele substitution is deleterious in a particular genetic background but is advantageous after substitutions have occurred in the genetic background (i.e. either in other domains of the same gene or in different genes). This is known as Dobzhansky-Muller (DM) incompatibility (reviewed in Welch, 2004). Originally introduced to explain the origin of reproductive isolation due to rendering alleles deleterious after confronting them with a different genetic background in hybrids (Orr, 1995; Orr and Turelli, 2001), recent empirical studies suggest a positive role of DM incompatibilities in evolution, because they allow selection for alleles which are deleterious in one genetic background after substitutions have occurred in the genetic background (“compensatory substitutions” in the genetic background allow for “compensated pathogenic deviations”) (e.g. Peixoto *et al.*, 1998; Liu *et al.*, 2001; Kondrashov *et al.*, 2002; Kulathinal *et al.*, 2004; DePristo *et al.*, 2005; Poelwijk *et al.*, 2007).

A particularly informative approach to the study of epistatic selection is the comparison of protein sequences with substitutions that are known to be pathogenic in a particular species. About 10% of all amino acid substitutions among species involve known pathogenic mutations, so-called Compensated Pathogenic Deviations (CPD). Surprisingly this fraction is independent of the phylogenetic distance of the comparison as well as the group of organisms compared (mammals: Kondrashov *et al.* 2002; insects: Kulathinal *et al.*, 2004). These results suggest, that the compensatory substitutions are driven by selection and that the probability of epistasis is relatively independent of the specific examples investigated. We suggest that this fact is consistent with the assumption in our model that the probability of epistasis  $E$  is not highly sensitive to the specific situation we are considering or the allele at a locus.

The studies of Kondrashov (2002) and Kulathinal (2004) further suggest that epistatic selection is a regular component of molecular evolution but they do not identify the other

interaction partners. The evolution of the other interaction partners needs to be documented in order to answer the question whether the interacting genes experience co-evolutionary bursts or not.

Our model differs from other models of correlated or compensatory evolution by assuming only adaptive substitutions and epistatic selection. To investigate whether co-evolutionary bursts exist requires the simultaneous analysis of genes known to interact. As mentioned above true co-evolutionary dynamics has been experimentally demonstrated only in a few cases (reviewed in Fraser 2006). Most recently, the molecular evolution of multiple interacting proteins was addressed by Presgraves and Stephan (2007) who investigated the evolution of six proteins from the nuclear pore complex (NPC).

The NPC came to the attention of evolutionary geneticist when it was shown that *Nup96*, a component of the nuclear pore sub-complex *Nup107*, participates in the epistatic interactions that cause hybrid incompatibility between *Drosophila melanogaster* and *D. simulans* (Presgraves et al., 2003). Presgraves and Stephan (2007) investigated *Nup96* and five other NPC proteins (two other proteins from the sub-complex *Nup107* and three nucleoporins) that are known to interact and found that all of them experienced an excess of non-synonymous substitutions in the relatively recent past. A lineage specific analysis of nucleotide substitutions showed that much of the differences arose by co-evolutionary bursts among most if not all these interacting proteins. The extent and rate of evolution detected by Presgraves and Stephan is much higher than expected by external selection alone, given that only 5 to 10% of the genes in the *Drosophila* genome show evidence of adaptive evolution, while in this case all 6 genes of this system do. The rate and pattern of sequence evolution seems to suggest that bursts of substitutions are driven entirely by selection, rather than a mode in which pairs of substitutions co-evolve through a combination of genetic drift and epistatic selection, i.e. where one mutation drifts to fixation

followed by epistatic selection on the compensatory mutation. It will be interesting to see more detailed studies about the evolution of interacting genes to assess whether co-evolutionary bursts, as predicted by our model, are a regular feature of genomic evolution.

Our present model only addresses epistatic effects between two loci, but it can be easily extended to multiple loci (M. Rorick, unpublished). In the multilocus case, different loci may or may not be linked by pairwise epistatic effects. Thus, the distribution of epistatic effects is determined by an interaction matrix, which defines for substitutions at each locus the prevalence  $E$  and strength  $k$  of epistatic effects at each of the other loci. The multilocus model will allow us to explore the role of modularity for co-evolutionary dynamics. From the results reported here for the two-locus-model we expect that a modular distribution of probabilities for epistatic interactions (e.g. when there are high epistatic interaction probabilities among loci A, B, C and among loci D, E, F but not between the two groups) should be reflected in a pattern of dissociated co-evolution (Schlosser, 2002, 2004) among loci: loci belonging to the same module should tend to co-evolve, whereas loci belonging to different modules should evolve independently. While this prediction is relatively trivial in case of strict modularity (with prevalent epistasis within modules and no epistasis between them), it will be interesting to explore co-evolutionary dynamics after modularity has been relaxed by various degrees (e.g. by allowing some degree of epistasis between modules, for instance few links with relatively high epistatic interaction probabilities or many links with low epistatic interaction probabilities).

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## Tables

**Table 1** : number of substitutions per locus until convergence to maximal fitness ranks.

E	k	Mean	Median	Std.dev.	Skewness	Kurtosis	Correl.AB	N(runs)
0	n/a	6.74	7	2.20	0.35	0.02	0.004	1000
1	2.1	15.23	12	11.33	1.55	3.34	0.868	995
1	3.1	14.49	12	10.51	1.47	2.68	0.859	992
1	4.1	13.24	10	9.87	1.88	6.46	0.852	995
1	5.1	13.31	11	9.80	1.78	4.76	0.852	995
1	6.1	13.02	10	9.48	1.71	4.30	0.840	995
0.8	2.1	12.17	10	8.82	1.66	4.07	0.823	998
0.6	2.1	10.03	8	7.13	1.87	5.37	0.759	1000
0.4	2.1	8.64	8	4.94	1.08	1.40	0.558	1000
0.2	2.1	7.40	7	3.62	1.06	2.36	0.248	998

**Table 2:** total duration until convergence to maximal fitness ranks.

E	k	Mean	Median	Std.dev.	Skewness	Kurtosis	N(runs)
0	n/a	15262	12375	11241	1.44	2.62	1000
1	2.1	19416	13339	18561	1.61	2.62	995

**Table 3** rate of substitutions (Subst./5000 time steps) until convergence to maximal fitness ranks

E	k	Mean	Median	Std.dev.	Skewness	Kurtosis	N(runs)
0	n/a	7.98	5.31	9.415	6.34	65.20	1000
1	2.1	16.73	9.74	22.14	4.47	28.42	995

**Table 4:** rate of substitutions (Subst./5000 time steps) in time windows of 5000 time steps

E	k	Mean	Median	Std.dev.	Skewness	Kurtosis	N (time windows)
0	n/a	4.18	1.0	10.10	0.95	-0.64	2562
1	2.1	7.40	2.0	2.02	1.73	3.17	3407

**Table 5:** waiting time between substitutions at different loci per run

	E	k	Mean	Median	Std.dev.	Skewness	Kurtosis	N(runs)
Average waiting times/run	0	n/a	1187.3	706.2	1416.1	2.68	9.56	1000
	1	2.1	409.6	165.1	890.4	7.87	94.73	993
Median of waiting times/run	0	n/a	266.2	111.3	547.4	8.86	130.79	1000
	1	2.1	100.5	31.0	481.7	13.53	204.28	993

**Table 6:** Correlations between substitution rates (Subst./500 time steps) in locus A and B in different time windows (500 time steps) of same run

E	k	time windows excluded	Mean	Median	Std.dev.	N(runs)
0	n/a	1	0.168	-0.020	0.286	527
		2	0.088	-0.037	0.252	461
		4	0.029	-0.040	0.202	283
1	2.1	1	0.574	0.643	0.262	552
		2	0.569	0.633	0.261	530
		4	0.558	0.641	0.271	504
1	3.1	1	0.565	0.606	0.234	541
1	4.1	1	0.535	0.586	0.262	548
1	5.1	1	0.494	0.524	0.248	580
1	6.1	1	0.470	0.506	0.258	570
0.8	2.1	1	0.530	0.586	0.277	506
0.6	2.1	1	0.444	0.469	0.281	463
0.4	2.1	1	0.365	0.395	0.295	470
0.2	2.1	1	0.247	0.486	0.300	467
0.5	2.1	1	0.404	0.432	0.294	432

**Table 7:** number of substitutions per locus until convergence to maximal fitness ranks for different probabilities of epistatic interactions and initial ranks.

rankA <sub>0</sub>	rankB <sub>0</sub>	E	k	MeanA	MedA	Std.dev.A	MeanB	MedB	Std.dev.B	Correl.AB	N(runs)
500	500	1	2.1	15.20	12	11.36	15.25	12	11.30	0.868	995
1000	500	1	2.1	13.75	10	12.84	15.67	12	12.81	0.895	992
500	500	1	4.1	13.16	11	9.79	13.32	10	9.96	0.852	995
1000	500	1	4.1	10.94	8	10.12	13.56	10	10.22	0.878	996
500	500	1	6.1	13.17	11	9.51	12.87	10	9.44	0.840	995
1000	500	1	6.1	9.82	7	8.97	12.72	10	9.11	0.862	990
500	500	0.8	2.1	12.19	10	9.15	12.15	10	8.48	0.823	998
1000	500	0.8	2.1	9.47	7	9.24	12.13	10	8.90	0.852	998
500	500	0.5	2.1	9.07	8	5.36	9.02	8	5.47	0.594	997
1000	500	0.5	2.1	5.42	4	5.31	8.86	7	5.30	0.659	999
500	500	0.2	2.1	7.40	7	3.62	7.41	7	3.62	0.248	998
1000	500	0.2	2.1	2.48	0.5	3.37	7.27	7	3.01	0.396	1000

## Figure legends

**Fig. 1** Overview of model. Each locus comprises  $n$  alleles, which all differ in fitness ranks. Fitness ranks of alleles can be ordered on a scale ranging from  $r=1$  to  $r=n$  (black bars). Mutation of one locus – for example the A locus as illustrated here - produces an allele of either lower or higher rank but only adaptive mutations to alleles of higher rank are fixed and result in the substitution of the old allele (black circle) of rank  $r_A$  by the new allele (grey circle) of rank  $r'_A$ . A substitution in the first locus has epistatic effects on the second locus – B in the case illustrated – with a certain probability  $E$ . Epistatic effects result in a displacement of the fitness rank of the current allele of the second locus (black circle) without substitution. We assume that negative epistatic effects (shifts to lower ranks as illustrated here) and positive epistatic effects (shifts to higher ranks) are equally likely and follow a distribution, whose variance and expected value are largely determined by a parameter  $k$  (see text for further explanation).

**Fig. 2** Distributions of epistatic effects. Ranks have been renormalized to the interval from 0 (minimal rank) to 1 (maximal rank). **A:** Diagrams depict different probability density functions (y axis) of ranks after epistatic effect (x axis) for three exemplary cases of current ranks (i.e. ranks before epistatic effect)  $r$  ( $r=0.5$ ,  $r=0.7$  and  $r=0.9$ ). Each diagram shows probability density functions for three different values of  $k$  illustrating that the variance increases with decreasing  $k$ . Note that per assumption of our model the current rank in each case is the median of the probability density function, that is increases or decreases in rank due to epistatic effects are equally likely. **B:** While the

probability of positive and negative epistatic effects remains the same with increasing  $r$ , average epistatic effects (i.e., the difference between mean rank after epistasis  $E(r')$  and current rank  $r$ ) are negative for  $r > 0.5$ . This is due to the fact that negative epistatic effects become on average larger and positive epistatic effects on average smaller with increasing  $r$  (see **A**).

**Fig. 3** Evolution of ranks and substitution events in two loci without epistatic interactions. Three exemplary runs are illustrated (**A**, **B**, **C**). Each example shows (from top to bottom): changes of ranks in locus A, substitution events in locus A (1 indicates that a substitution took place, 0 that no substitution took place), changes of ranks in locus B, substitution events in locus B. Only the uppermost ranks (900-1000) are shown. Evolutionary trajectories of ranks and substitution events are only shown up to the point when both loci converge onto maximal rank thus preventing further evolution. Note that in the absence of epistasis ranks can only increase. The trajectories of the two loci in a single run are not similar.

**Fig. 4** Evolution of ranks and substitution events in two loci with prevalent and strong epistatic interactions ( $E=1$ ,  $k=2.1$ ). Three exemplary runs are illustrated (**A**, **B**, **C**). Each example shows (from top to bottom): changes of ranks in locus A, substitution events in locus A (1 indicates that a substitution took place, 0 that no substitution took place), changes of ranks in locus B, substitution events in locus B. Again, only the uppermost ranks (900-1000) are shown. Evolutionary trajectories of ranks and substitution events are only shown up to the point when both loci converge onto maximal rank thus preventing further evolution. Note that in the presence of epistasis ranks can increase or



decrease. The trajectories of the two loci in a single run are similar and are characterized by simultaneous coevolutionary bursts. Beginning and end of each burst is indicated by arrows and arrowheads, respectively. For the beginning of each run, only the end of the initial climb of ranks is marked with an arrowhead. In the rank diagrams beginning and end of each burst is indicated for both loci, but in substitution diagrams only the substitution in the locus, which in fact initiates or terminates a particular coevolutionary burst is labeled with arrows and arrowheads, respectively. See text for details.

**Fig. 5** Distribution of total number of substitutions until reaching maximal rank per locus in runs without epistasis (hatched line) or with prevalent and strong epistatic interactions (solid line;  $E=1$ ,  $k=2.1$ ). Note the higher average number of substitutions and higher variance in epistatic runs compared to non-epistatic runs. Diagrams are based on 995 runs in the epistatic case and 1000 runs in the nonepistatic case (because both loci are considered separately, this results in 1990 run-values for the epistatic case and 2000 run-values for the non-epistatic case).

**Fig. 6:** Scatterplots of total number of substitutions until reaching maximal rank in locus A vs. locus B in runs without epistasis (**A**) or with prevalent and strong epistatic interactions ( $E=1$ ,  $k=2.1$ ) (**B**). Note that total substitution numbers are highly correlated between the two loci in case of epistasis ( $\psi=0.868$ ) but are uncorrelated in absence of epistasis ( $\psi=0.004$ ). Diagrams are based on 995 runs in the epistatic case and 1000 runs in the nonepistatic case.

**Fig. 7** Distribution of rates of substitutions in both loci until reaching maximal rank in runs without epistasis or with prevalent and strong epistatic interactions ( $E=1$ ,  $k=2.1$ ). Diagrams are based on 995 runs in the epistatic case and 1000 runs in the nonepistatic case. **A:** Total rates of substitution (plotted as natural logarithm of total number of substitutions in both loci/duration of run until both loci have reached maximal rank) are on average slightly higher for the epistatic case. **B:** Number of substitutions in both loci per time window of 5000 iterations (terminal time windows in which maximal ranks were reached in less than 5000 iterations were not included in the analysis). Both distributions show a peak at 0-2 substitutions/5000 time steps reflecting long periods of stasis when loci have reached near-maximal rank. Non-epistatic runs show a second peak centered around 10-16 substitutions/5000 time steps, which is due to the initial climb to near-maximal ranks. Epistatic runs show a more even distribution of rates due to positive (arrow 1) and negative epistatic effects (arrows 2,3) as discussed in more detail in the text.

**Fig. 8:** Distribution of correlations between substitution rates (Subst./500 time steps) in locus A and B in different time windows of the same run for runs without epistasis or with prevalent and strong epistatic interactions ( $E=1$ ,  $k=2.1$ ). Each time window was 500 time steps long. Because correlations can only be meaningfully calculated if a sufficient number of datapoints are available, only runs that covered at least 20 time windows in total were included in the analysis. In order to avoid artefacts, the first time window (**A**) or the first four time windows (**B**) were excluded from the analysis. Upper panels: Distribution of correlations between substitution rates. Lower panels: Cumulative density

functions of upper panels. Grey lines indicate the corresponding normal distribution approximations. The 95% percentiles (based on the normal distribution approximations) are indicated.

**Fig. 9:** Effect of strength and prevalence of epistasis on coevolution of two loci.

Coevolution is measured either by the correlation between total number of substitutions until reaching maximal rank in locus A vs. locus B of the same run (**A,C**) or by the mean correlations between rates of substitutions in locus A vs. locus B in different time windows (of 500 time steps) of the same run (**B,D**; bars indicate standard error of mean).

As in Figure 8A only runs that covered at least 20 time windows were included in the analysis and the first time window was discarded. **A,B:** Strength of epistasis: All

simulations had prevalent epistasis ( $E=1$ ) but varied regarding strength of epistasis  $k$ .

Varying the strength of epistasis has little effect on the coevolution of loci. **C,D:**

Prevalence of epistasis: All simulations had strong epistatic effects ( $k=2.1$ ) but varied with respect to prevalence of epistasis  $E$  (i.e. the probability that a substitution in one locus has epistatic effects on the other locus). The probability of both loci to coevolve is strongly dependent on the prevalence of epistasis.

**Fig. 10:** Effect of differences in initial ranks on coevolution of two loci. Initial conditions

were either highly asymmetrical with locus A starting from maximal rank 1000 and locus B from an intermediate rank of 500 or symmetrical with both loci starting at an

intermediate rank of 500. **A,B:** Correlations between total number of substitutions until reaching maximal rank in locus A vs. locus B of the same run under different conditions

of epistatic strength  $k$  (**A**) or prevalence  $E$  (**B**). Simulations with asymmetrical initial conditions (grey squares) do not differ greatly from simulations starting with symmetrical initial conditions (black circles). However, with decreasing prevalence of epistasis, correlations get increasingly higher for the asymmetric initial conditions. **C,D**: Detailed comparison between symmetrical and asymmetrical initial conditions for  $E=1$  (**C**) and  $E=0.2$  (**D**). Upper panels: Distribution of total number of substitutions until reaching maximal rank per locus in runs with symmetrical (hatched black line) or asymmetrical (solid black line) initial conditions. For the asymmetrical conditions, the distribution of substitutions in locus A and locus B are also plotted individually (light and dark grey lines, respectively). Note that with decreasing prevalence of  $E$ , the distribution for locus A shifts towards zero or very small substitution numbers reflecting decreasing probabilities for escape from maximal rank due to epistasis. Middle and lower panels: Scatterplots of total number of substitutions until reaching maximal rank in locus A vs. locus B in runs with symmetrical (middle panel) and asymmetrical (lower panel) initial conditions.

Fig. 1

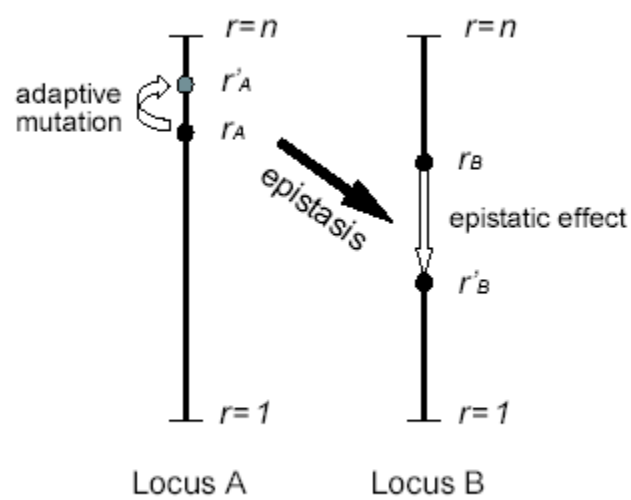


Fig. 2

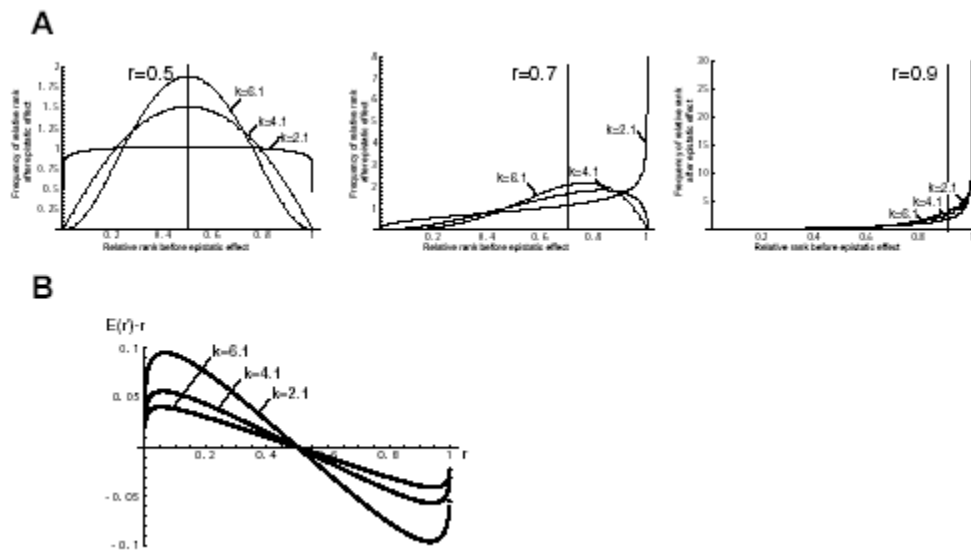
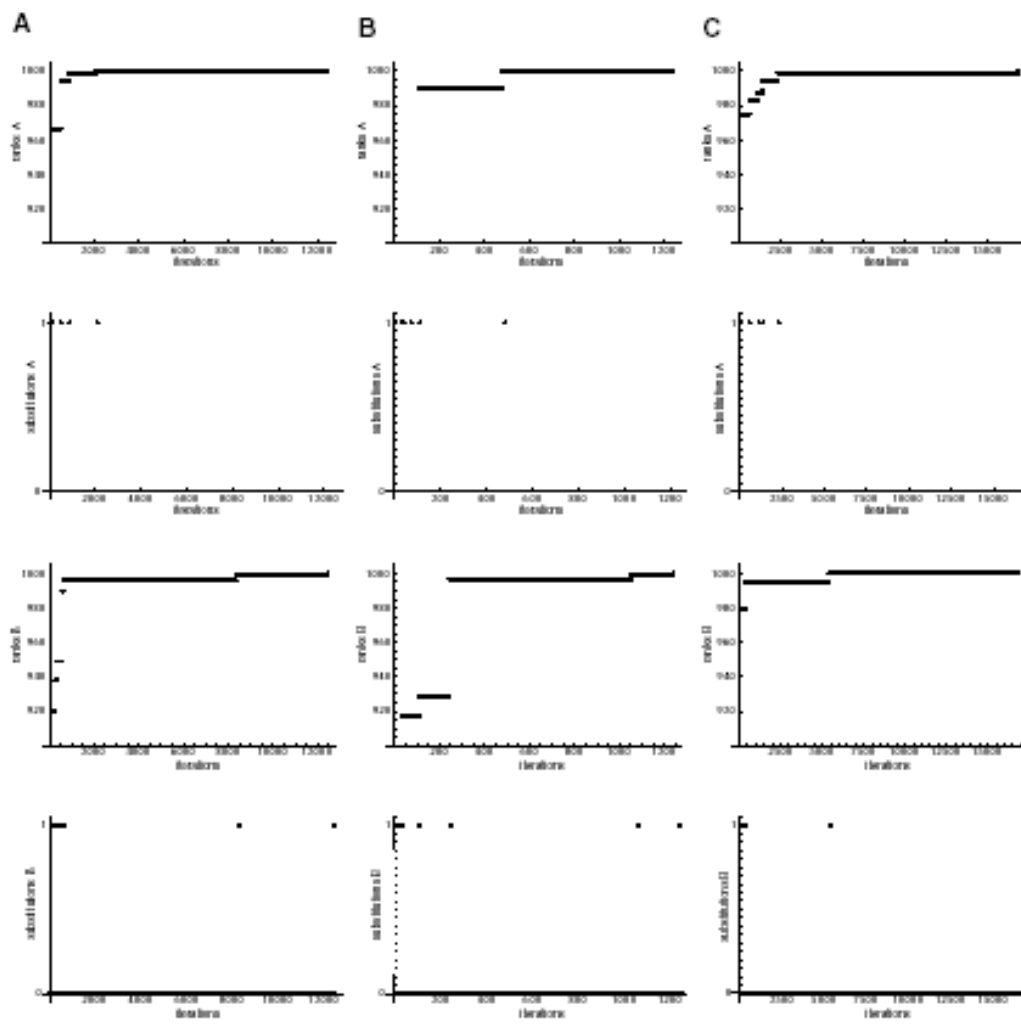


Fig. 3



AC

Fig. 4

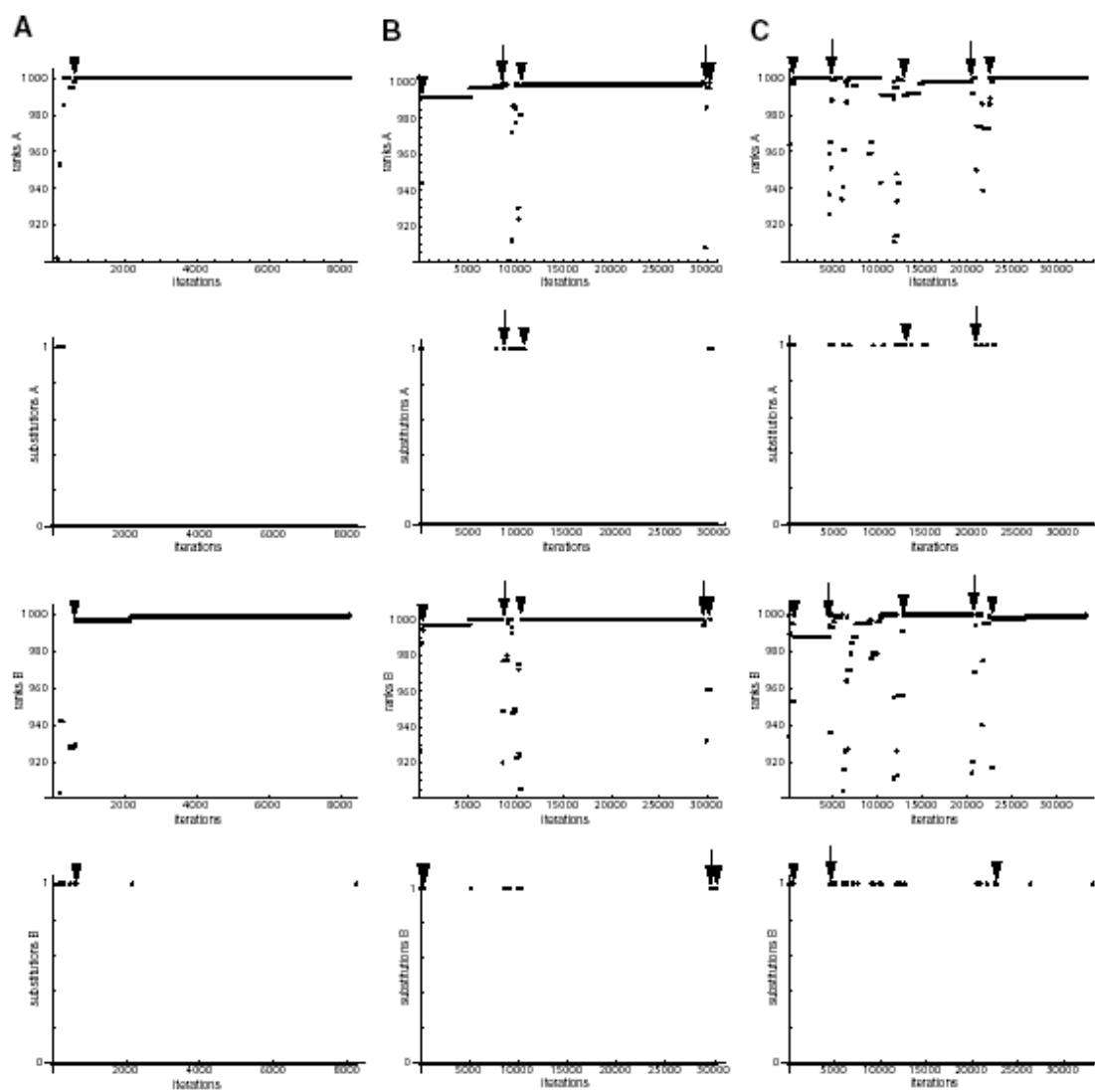
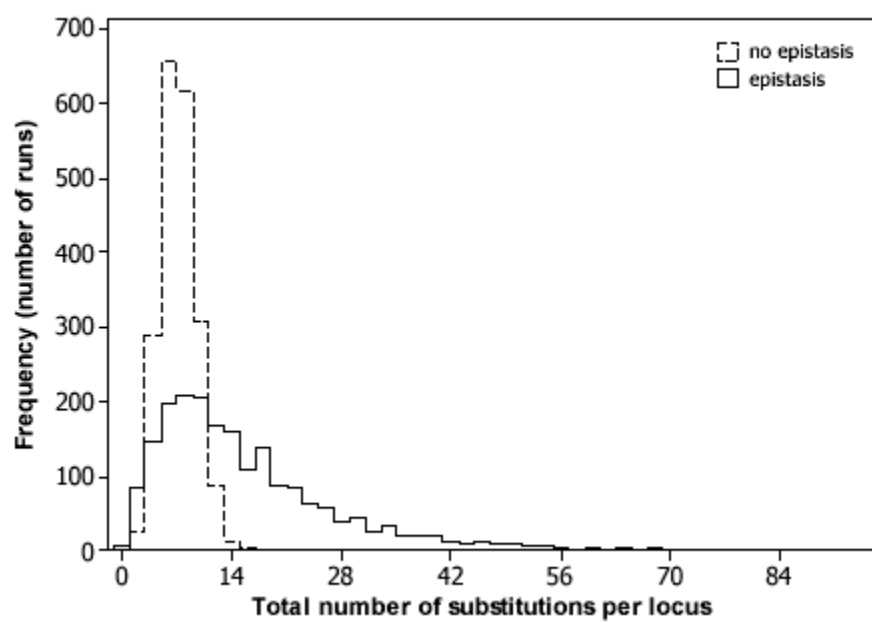




Fig. 5



Accepted man.

Fig. 6

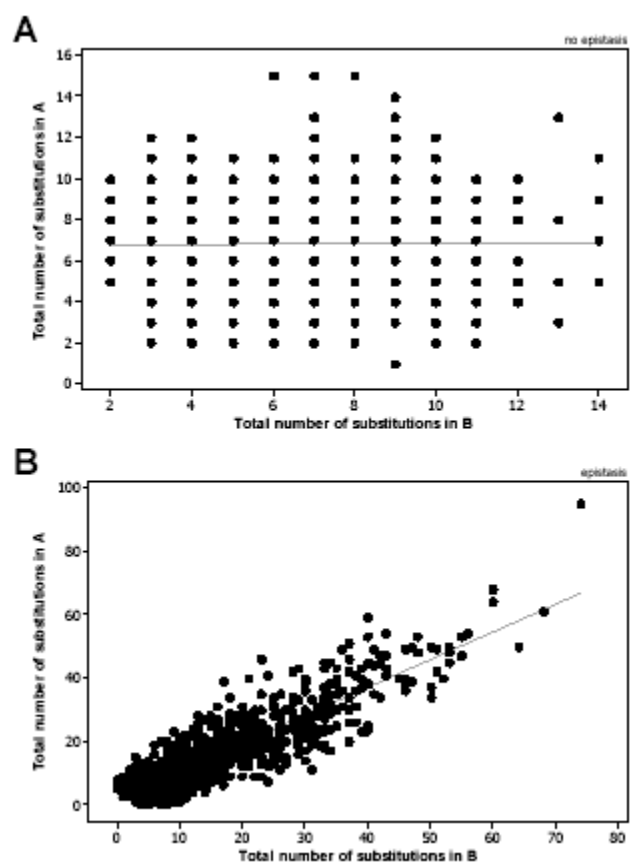


Fig. 7

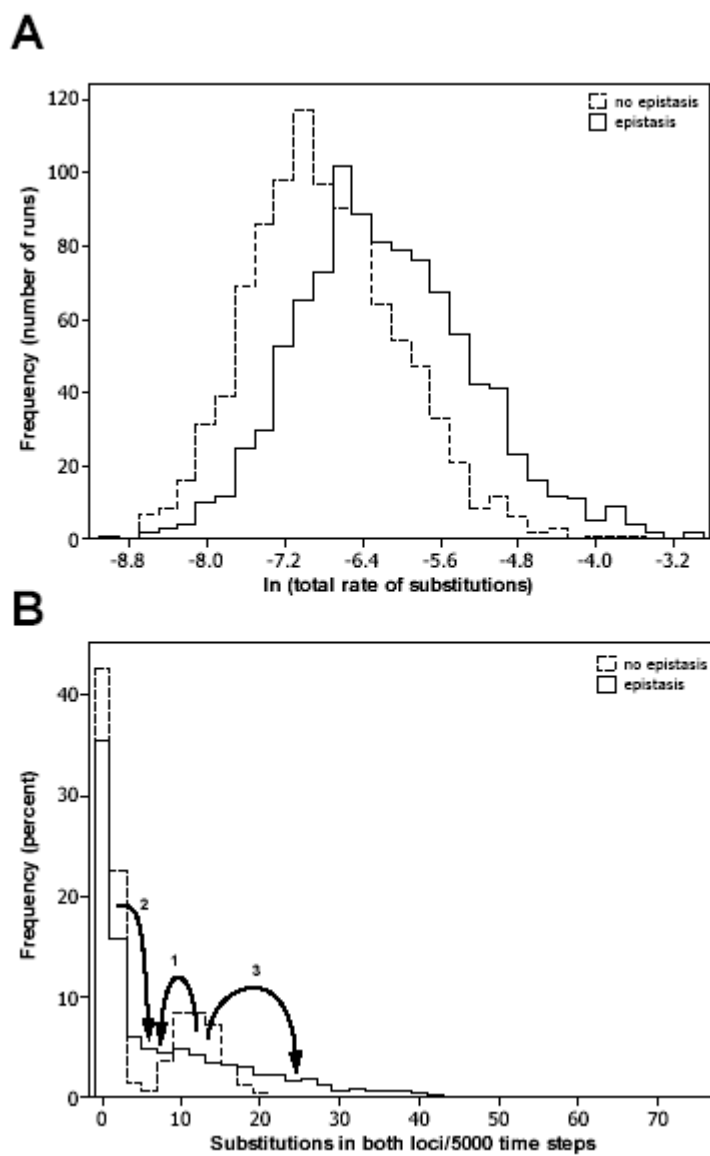


Fig. 8

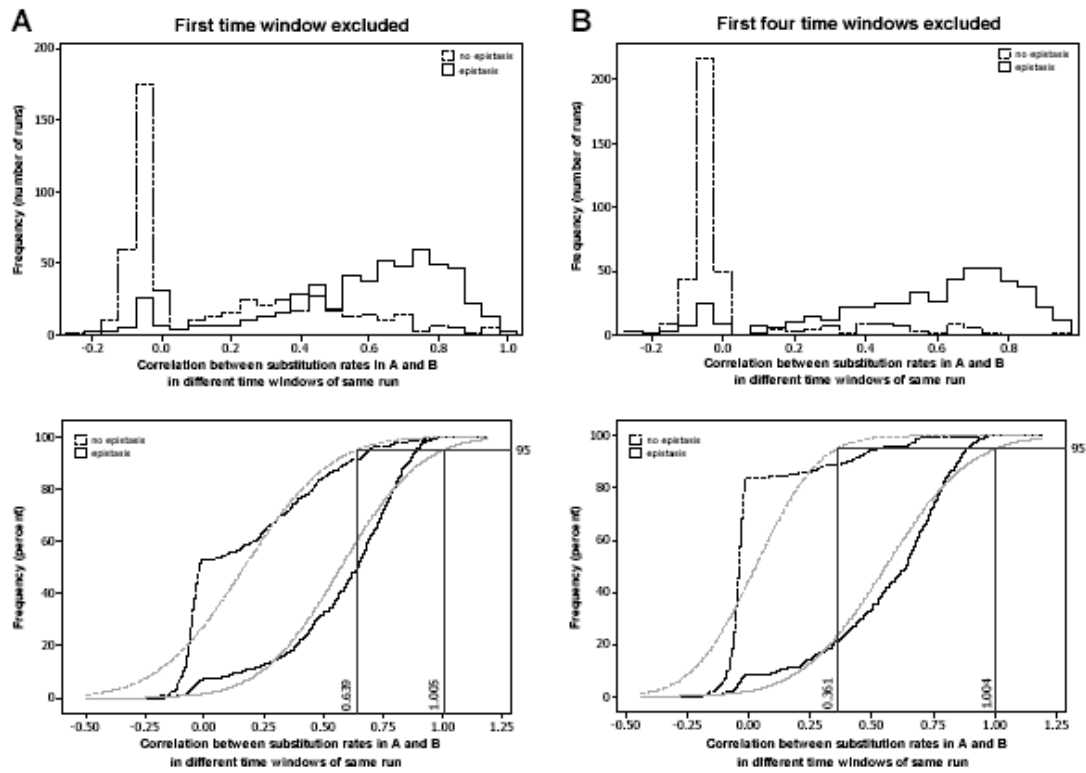


Fig. 9

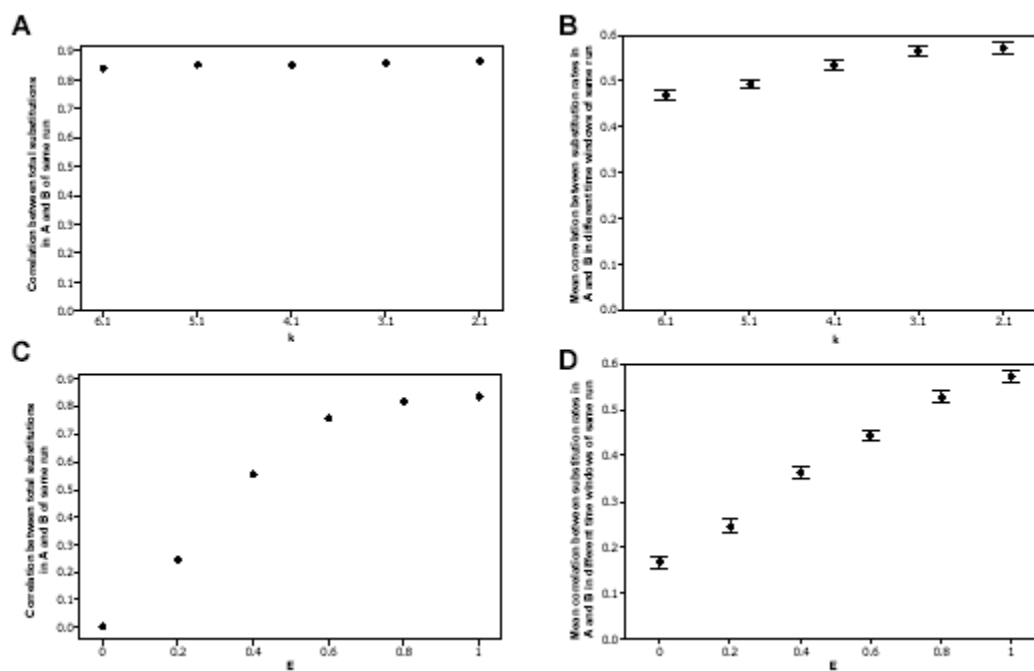


Fig. 10

