The Role of Recombination in Evolutionary Rescue

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ABSTRACT How likely is it that a population escapes extinction through adaptive evolution? The answer to this question is of great relevance in conservation biology, where we aim at species' rescue and the maintenance of biodiversity, and in agriculture and medicine, where we seek to hamper the emergence of pesticide or drug resistance. By reshuffling the genome, recombination has two antagonistic effects on the probability of evolutionary rescue: it generates and it breaks up favorable gene combinations. Which of the two effects prevails depends on the fitness effects of mutations and on the impact of stochasticity on the allele frequencies. In this article, we analyze a mathematical model for rescue after a sudden environmental change when adaptation is contingent on mutations at two loci. The analysis reveals a complex nonlinear dependence of population survival on recombination. We moreover find that, counterintuitively, a fast eradication of the wild type can promote rescue in the presence of recombination. The model also shows that two-step rescue is not unlikely to happen and can even be more likely than single-step rescue (where adaptation relies on a single mutation), depending on the circumstances.

KEYWORDS rapid adaptation; epistasis; drift; population dynamics; ecology

POPULATIONS facing severe environmental change need to adapt rapidly to the new conditions, or they will go extinct. The most prominent examples for evolutionary rescue in natural populations are provided by failed eradication of pathogens or pests that develop resistance against drugs or pesticides. Understanding which factors drive the evolution of resistance has been a concern since the application of drugs and pesticides. In recent years, the topic of evolutionary rescue has attracted increasing interest of evolutionary biologists at a broader front. Both theoretical models and laboratory experiments have been used to investigate the influence of many genetic or environmental factors on the survival probability of an endangered population, *e.g.*, the importance of standing genetic variation, sexual reproduction, the history of stress, the severity and speed of environmental deterioration, or population structure (Bell and Collins 2008; Orr and Unckless 2008, 2014; Bell and Gonzalez 2009, 2011; Agashe *et al.* 2011; Lachapelle and Bell 2012; Gonzalez and Bell 2013; Uecker *et al.* 2014; see also the reviews by Alexander *et al.* 2014 and Carlson *et al.* 2014). Despite significant progress, a largely open area in research on rescue concerns the influence of recombination on the probability of population survival.

Recombination has two fundamental effects on adaptation that work against each other: it brings favorable gene combinations together but it also breaks them up. Recombination hence has the potential both to promote rescue and to impede it. In classical population genetics (assuming a constant population size), the interplay of the two opposing effects of recombination has been an intensively studied problem for decades (*e.g.*, reviewed in Barton and Charlesworth 1998; Otto 2009; Hartfield and Keightley 2012). Fundamentally, recombination acts to reduce the linkage disequilibrium between alleles. For two loci with two alleles each, recombination increases the number of double mutants if the linkage disequilibrium (LD) between the mutant alleles is negative; it decreases them when LD is positive; and it has no effect if the loci are in linkage equilibrium. A major source of linkage disequilibria is epistasis, with negative epistasis leading to negative LD and positive epistasis leading to positive LD (Felsenstein 1965; Kouyos *et al.* 2009). For evolutionary rescue, the shift of the environment from original to perturbed conditions may lead to a change in epistasis during the course of evolution, which adds new aspects to the problem of how recombination affects adaptation.

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Combination drug therapy (as well as the use of herbicide mixtures in agriculture) seeks to limit the evolution of resistance by increasing the number of mutations that are required to restore fitness above one. In the absence of drugs, resistant types often suffer a selective disadvantage; upon treatment, the fitness landscape changes. Experimental studies show that depending on the drug combination, mutants resistant to one of the drugs can display a lower or a higher fitness than the fully sensitive strain (*e.g.*, Chait *et al.* 2007); *i.e.*, a whole range of fitness landscapes and epistatic interactions before and after the start of treatment are displayed by pathogens. Understanding under which conditions recombination can undermine the strategy of combination therapy is of great relevance to plan a successful treatment. Consequently, the effect of recombination on the evolution of drug resistance has attracted considerable attention from epidemiologists, in particular with respect to resistance in the human immunodeficiency virus (HIV) (Bretscher *et al.* 2004; Fraser 2005; Carvajal-Rodríguez *et al.* 2007; Kouyos *et al.* 2009). However, most epidemiological models are deterministic and focus on the time to resistance rather than on the probability of resistance (Bretscher *et al.* 2004; Fraser 2005). An exception is the simulation study by Kouyos *et al.* (2009), which incorporates stochasticity and allows populations to go extinct. Their study demonstrates how the population dynamics affect the emergence of linkage disequilibria and hence the influence of recombination on the probability of and time to resistance, finding that recombination usually slows down the evolution of resistance. However, the model is specific to the complex epidemiological dynamics of HIV and cannot be used to draw general conclusions about the role of recombination in evolutionary rescue.

In a general evolutionary context, theoretical models of rescue where population genetics and population dynamics are intertwined have mainly followed two routes. In one class of models, adaptation relies on changes at just a single locus, and recombination consequently does not appear (Gomulkiewicz and Holt 1995; Iwasa *et al.* 2003, 2004; Bell and Collins 2008; Orr and Unckless 2008, 2014; Martin *et al.* 2013; Uecker *et al.* 2014). The second class of models, motivated by conservation biology, is based on a quantitative genetics approach where (infinitely) many loci of small effect determine the fitness of an organism (Pease *et al.* 1989; Lynch *et al.* 1991; Bürger and Lynch 1995; Lande and Shannon 1996; Polechová *et al.* 2009; Duputié *et al.* 2012). These models usually do not incorporate an explicit genetic architecture that would allow for the investigation of the effect of recombination or assume linkage equilibrium. In their review, Carlson *et al.* (2014) refer to a single study—Schiffers *et al.* (2013)—for the effect of linkage on the probability of rescue. In their simulation study of an explicit multilocus model, Schiffers *et al.* (2013) compare rescue probabilities for the two extreme cases of complete linkage and free recombination. In contrast to Kouyos *et al.* (2009), they find that linkage significantly decreases the probability of rescue; the related follow-up study by Bourne *et al.* (2014) that allows for intermediate linkage comes to the same conclusion. However, both models are tailored to consider a highly specific ecological situation of climate change in a spatially structured environment. Just as in the study by Kouyos *et al.* (2009), they are not designed to serve as a baseline model.

In this article, we set up and analyze a generic two-locus model for the role of recombination in evolutionary rescue. A population experiences a sudden severe environmental change; adaptation relies on two mutations and can happen either from the standing genetic variation or from *de novo* mutations. There are hence two phases—the time before and the time after the environmental change—during which recombination acts to increase or decrease the chances of population survival, depending on the fitness scheme and the strength of drift. We provide an accurate analytical framework based on branching process theory, complemented by computer simulations, to obtain an intuitive understanding of the principles underlying rescue under these conditions. We conclude with two notable observations that might contradict spontaneous intuition and that could be of practical relevance.

The Model

Consider a panmictic population of variable size $N = N(t)$ that faces the risk of extinction after a sudden environmental change. Individuals are haploid during their selective phase. Their fitness before and after the change depends on two loci with two alleles each such that there are four genotypes: the wild-type ab, single-mutant types Ab and aB, and the double-mutant *rescue type* AB. Each generation, each individual produces a large number X of gametes. Mutations happen with probability u at each locus and in both directions. Gametes form diploid zygotes, which produce haploid offspring. The recombination probability between the two loci is r. Thereafter, selection takes place. By n_{ab}, n_{Ab}, n_{aB}, and n_{AB}, we denote the number of the respective genotypes in the population; hence $N = n_{ab} + n_{Ab} + n_{aB} + n_{AB}$. We obtain for the number of haploids after reproduction but before selection

$$\begin{aligned}
\nu_{ab}X &:= \big(\hat{n}_{ab} - r\hat{D}N\big)X,\\
\nu_{Ab}X &:= \big(\hat{n}_{Ab} + r\hat{D}N\big)X,\\
\nu_{aB}X &:= \big(\hat{n}_{aB} + r\hat{D}N\big)X,\\
\nu_{AB}X &:= \big(\hat{n}_{AB} - r\hat{D}N\big)X
\end{aligned} \tag{1}$$

with the proportion of each genotype after mutation but before recombination

$$\begin{aligned}
\hat{n}_{ab} &:= (1-u)^2 n_{ab} + (1-u)u n_{Ab} + (1-u)u n_{aB} + u^2 n_{AB},\\
\hat{n}_{Ab} &:= u(1-u)n_{ab} + (1-u)^2 n_{Ab} + u^2 n_{aB} + (1-u)u n_{AB},\\
\hat{n}_{aB} &:= u(1-u)n_{ab} + (1-u)^2 n_{aB} + u^2 n_{Ab} + (1-u)u n_{AB},\\
\hat{n}_{AB} &:= u^2 n_{ab} + (1-u)u n_{Ab} + (1-u)u n_{aB} + (1-u)^2 n_{AB}
\end{aligned} \tag{2}$$

and the linkage disequilibrium (after mutation)

$$\hat{D} = \frac{1}{N^2}(\hat{n}_{ab}\hat{n}_{AB} - \hat{n}_{Ab}\hat{n}_{aB}). \quad (3)$$

Before the environmental change, the population is well adapted to its environment and the population size is constant, $N(t) = N_0$. The numbers of the respective genotypes in the new generation are determined by multinomial sampling of N_0 individuals, where the probability to sample an individual of type i ($i \in \{ab, Ab, aB, AB\}$) is given by

$$\frac{(1+\sigma_i)\nu_i}{\sum_i (1+\sigma_i)\nu_i}. \quad (4)$$

The selection coefficients σ_i quantify selection before the environmental change. We set $\sigma_{ab} = 0$ and assume that all mutants are deleterious relative to the wild type, σ_{Ab}, σ_{aB}, $\sigma_{AB} < 0$. After the switch in the environment, the population size is variable and will usually initially decline. Individuals of type i survive with probability $(1+s_i)/X$ such that their number after selection is Poisson distributed with parameter

$$\nu_i(1+s_i). \quad (5)$$

The s_i parameterize the expected growth (for $s_i > 0$) or decline ($s_i < 0$) of a type-i population after the environmental change. Usually, we assume that only the rescue type can grow under these conditions ($s_{AB} > 0$), and s_{ab}, s_{Ab}, and s_{aB} are all < 0. Epistasis before and after the environmental change is measured as the deviation of fitnesses from multiplicativity; *i.e.*,

$$\begin{aligned} E_1 &:= (1+\sigma_{ab})(1+\sigma_{AB}) - (1+\sigma_{Ab})(1+\sigma_{aB}) \\ &= \sigma_{AB} - (\sigma_{Ab} + \sigma_{aB} + \sigma_{Ab}\sigma_{aB}), \\ E_2 &:= (1+s_{ab})(1+s_{AB}) - (1+s_{Ab})(1+s_{aB}), \end{aligned} \quad (6)$$

respectively (see Kouyos *et al.* 2009 for the definition of epistasis in discrete-time *vs.* continuous-time models). If the total number of individuals after selection is $> N_0$, it is reduced to N_0. Density regulation hence occurs via a hard carrying capacity, and there is no density dependence for $N \leq N_0$.

The simulations follow this scheme. We start with a population that is entirely composed of wild-type individuals and let it evolve for a large number of generations such that mutation–selection balance is reached before the environment changes (increasing the number of generations did not influence the results). We follow the population dynamics after the environmental change either until the population has gone extinct or until the number of *AB* mutants has grown to 90% carrying capacity and the population can be considered as rescued (in a few cases, we modify the criterion for "rescue" to reduce the simulation time; this is then explicitly stated). The simulation code is written in the *C* programming language, making use of the *GNU Scientific Library* (Galassi *et al.* 2009).

Analysis and Results

Our analytical approach to estimate the rescue probability combines deterministic and stochastic aspects. We focus on populations that are initially large and (mostly) describe the dynamics of the wild type and the single mutants deterministically. Even if the initial population size is large, however, the number of rescue type individuals (*AB* double mutants) is potentially small and subject to strong stochasticity. These stochastic dynamics depend on all genotype frequencies in the population, which typically change over time in response to environmental change and selection. We address the stochasticity in the number of *AB* mutants by means of branching process theory. The basic mathematical ingredients used are summarized in Supporting Information, File S1; File S2 and File S3 contain the derivations of our main results. In File S4, we briefly test the limits of our approximations. Since selection is potentially strong, details of the life cycle need to be taken into account to arrive at quantitatively accurate analytical predictions. We take care of these details in the supporting information but neglect them in the main text below, where we summarize our main results.

The probability of evolutionary rescue depends on two factors: the number of rescue types that are generated and their establishment probability in the population after the environmental change. Both quantities are affected by recombination. Mutant genotypes can either be present in the population prior to the switch in the environment or newly arise during population decline. Double mutants, in particular, can be generated either by mutation from single mutants with a constant probability per individual or by recombination of two single mutants with a probability that depends on the (time-dependent) genotype composition in the population. Which route of rescue is most relevant depends on the model parameters for mutation, selection, recombination, and drift. In this section, we progressively describe all routes to rescue. We start with a scenario where single mutants are lethal in the new environment. In this case, rescue entirely relies on double mutants from the standing genetic variation (we assume throughout the analysis that the mutation probability is so small that a direct transition from the wild type to the double mutant can be neglected). Next, we assume that one of the single mutants is sufficiently viable in the new environment that it can still generate the rescue type by mutation after the environmental change. In both of these scenarios, recombination can be beneficial or detrimental in the old environment due to its effect on the number of rescue genotypes in the standing genetic variation; its effect after the change in the environment is, however, always detrimental (recombination with the wild type reduces the establishment probability of the rescue type). This is different if both single mutants are viable under the new conditions; in that case, recombination increases the rate at which the rescue type is generated after the environmental change, and—depending on the fitness scheme—this can outweigh the negative effect of recombination. Finally, if single mutants have a fitness larger than one, formation of the double mutant is not required for rescue. We briefly discuss when it is appropriate to neglect its generation in this case. The fitness schemes used in the following four sections are summarized in Table 1.

Scheme 1: Single mutants are lethal in the new environment

Within our first scheme, we assume that single mutants are lethal in the new environment ($s_{Ab} = s_{aB} = -1$). This means that *de novo* generation of the rescue type is prevented after the change in the environment and rescue—if it happens—happens from double mutants in the standing genetic variation.

Before the change in the environment, single mutants segregate in the population at mutation–selection balance, which we approximate deterministically as constant in time, $\overline{n}_{Ab} \approx -uN_0/\sigma_{Ab}$, $\overline{n}_{aB} \approx -uN_0/\sigma_{aB}$ (ignoring the influence of recombination on the frequency of single mutants). Double mutants are generated from single mutants by either recombination or mutation, at a total rate of $\sim u(\overline{n}_{Ab} + \overline{n}_{aB}) + r(\overline{n}_{Ab}\overline{n}_{aB}/N_0)$. In the presence of wild-type individuals, the double mutant suffers from recombination with the wild type and gets broken up at rate $\sim (n_{ab}/N_0)r \approx r$, leading to an "effective fitness" of $\approx 1 + \sigma_{AB} - r < 1$. Using this, we can describe the number of *AB* mutants in the standing genetic variation, n_{AB} by a subcritical branching process with immigration (see File S1, section S1.2).

The establishment probability of the rescue type after the environmental change depends on the dynamics of the wild type. If the wild type is lethal ($s_{ab} = -1$), *AB* mutants are not broken up by recombination and establish with probability $p_{\text{est}}^{(AB)} \approx 2s_{AB}$ (Haldane 1927). If the wild type disappears slowly, with expected extinction time much larger than the typical establishment time of a rescue type, the growth parameter of a rare rescue type is $\sim s_{AB} - r$ and we can approximate $p_{\text{est}}^{(AB)} \approx 2\max[(s_{AB} - r), 0]$. In an intermediate parameter range, where the establishment time of the rescue type and the extinction time of the wild type cannot be separated, we need a more refined approximation (see File S1, Equation S1.29): we derive this approximation by treating the wild-type extinction time as a stochastic variable, whose distribution can be estimated. Conditioned on this extinction time, the establishment probability of the rescue type follows from a time-dependent branching process (Uecker and Hermisson 2011). Finally, for a given n_{AB} at the time of environmental change, the probability of evolutionary rescue follows as

$$P_{\text{rescue}}(n_{AB}) \approx 1 - e^{-n_{AB}p_{\text{est}}^{(AB)}}, \tag{7}$$

which needs to be averaged over the distribution of n_{AB}.

Figure 1 shows the probability of evolutionary rescue for the three possible fitness schemes prior to the environmental change: no epistasis, negative epistasis, and positive epistasis. After the environmental change, epistasis is positive (or zero) since single mutants are lethal. Figure 1, A–C, shows the behavior for a very large population size ($N_0 = 10^8$), where all genotype frequencies prior to the environmental change are close to deterministic; in Figure 1, D–F, the population size is two orders of magnitude smaller ($N_0 = 10^6$), and stochasticity in the number of double mutants becomes important (see below).

Table 1 Fitness schemes used in *Analysis and Results*

Genotype	Scheme 1	Scheme 2	Scheme 3		Scheme 4
			Scenario 1	Scenario 2	
ab	$s_{ab} < 0$	$s_{ab} < 0$	Lethal	$s \le s_{ab} < 0$	Lethal
Ab	Lethal	$s_{Ab} < 0$	$s_{Ab} = s < 0$	$s_{Ab} = s < 0$	$s_{Ab} = s > 0$
aB	Lethal	Lethal	$s_{aB} = s < 0$	$s_{aB} = s < 0$	$s_{aB} = s > 0$
AB	$s_{AB} > 0$	$s_{AB} > 0$	$s_{AB} > 0$	$s_{AB} > 0$	$s_{AB} > s$

If the wild type is lethal in the new environment (Figure 1, solid circles), recombination affects the probability of rescue only via its effect on the standing genetic variation, and it is instructive to consider this case first. In terms of drug therapy, a lethal wild type constitutes an extreme form of the "hit hard" strategy; *i.e.*, drug dosage is chosen high to achieve a fast decline of the pathogen population. In File S3, section S3.1, we derive an approximation for rescue if we can treat all genotype frequencies in the standing genetic variation deterministically (see File S3, Equation S3.4); with $\sigma_{Ab} = \sigma_{aB} = \sigma$ and $\sigma_{AB} = (\sigma_{Ab} + \sigma_{aB} + \sigma_{Ab}\sigma_{aB}) + E_1$, and assuming that all selection coefficients are small, we obtain

$$P_{\text{rescue}}^{\text{det}} \approx 1 - e^{-2s_{AB}\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1}}. \tag{8}$$

From this, we can read that for $E_1 = 0$ (no epistasis, Figure 1A), the rescue probability is independent of recombination; for $E_1 < 0$ (negative epistasis, Figure 1B), it increases with r; and for $E_1 > 0$ (positive epistasis, Figure 1C), it decreases with r. For $r = 0$, the rescue probability depends strongly on epistasis $\left(P_{\text{rescue}}^{\text{det}} \approx 1 - e^{-2s_{AB}\frac{u^2N_0}{\sigma^2}\frac{2\sigma}{2\sigma+E_1}}\right)$, while for $r \gg \max[|2\sigma|, |2\sigma + E_1|]$, the rescue probability becomes independent of epistasis $\left(P_{\text{rescue}}^{\text{det}} \approx 1 - e^{-2s_{AB}\frac{u^2N_0}{\sigma^2}}\right)$ This is expected from deterministic theory: without epistasis, the population is in linkage equilibrium and recombination has no effect. Negative epistasis leads to negative linkage LD and recombination is favorable since it increases the number of *AB* mutants. Vice versa, positive epistasis leads to positive linkage disequilibrium and recombination is deleterious since it decreases the number of *AB* mutants (Felsenstein 1965). For sufficiently strong recombination, the population approaches linkage equilibrium irrespective of epistasis.

In Figure 1, D–F, population size is smaller by two orders of magnitude. The growth rate of the rescue genotype is two orders of magnitude larger such that $s_{AB}N_0$ is the same as in Figure 1, A–C. The deterministic prediction for the rescue probability (Equation 8) is hence unchanged. However, although the population size before the decline contains 10^6 individuals, stochastic fluctuations in the number of double mutants n_{AB} become important in this regime. Symmetric fluctuations in n_{AB} do not have symmetric effects on rescue,

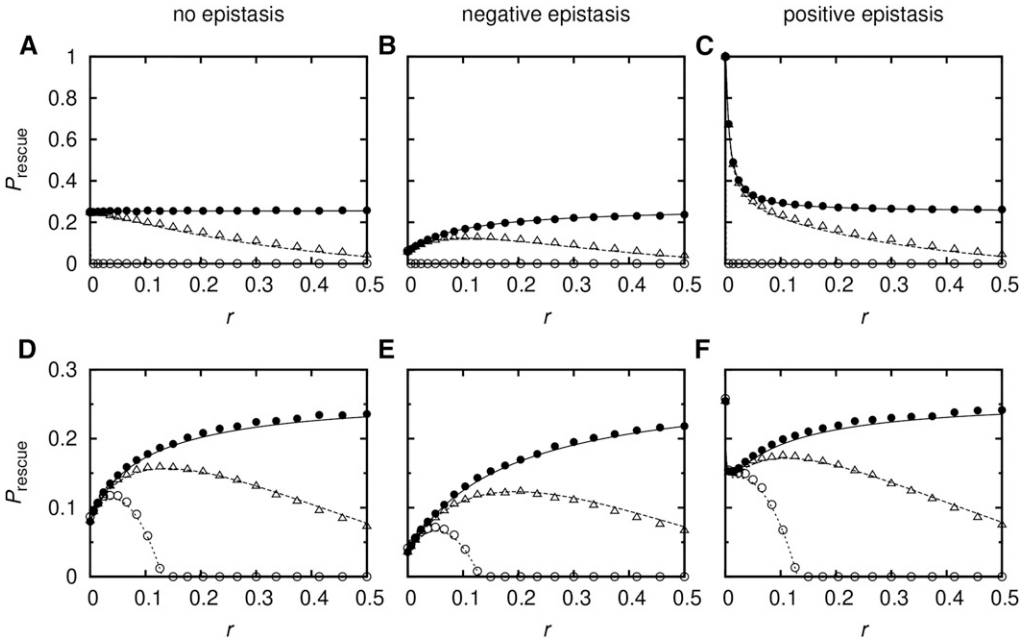

Figure 1 Probability of evolutionary rescue as a function of recombination when single mutants are lethal in the new environment. Solid circles correspond to an instantaneous elimination of the wild type ($s_{ab} = -1$), triangles to an extremely fast decay of the wild-type population size ($s_{ab} = -0.99$), and open circles to a slow decay of the wild-type population size ($s_{ab} = -0.005$). Before the switch in the environment, selection against the single mutants is $\sigma_{Ab} = \sigma_{aB} = -0.01$, and epistasis is absent (A and D: $\sigma_{AB} = -0.0199$; *i.e.*, $E_1 = 0$), negative (B and E: $\sigma_{AB} = -0.1$; *i.e.*, $E_1 \approx -0.08$), and positive (C and F: $\sigma_{AB} = -0.0001$; *i.e.*, $E_1 \approx 0.02$). The other parameter values are $u = 10^{-5}$, $s_{AB} = 0.0015$, and $N_0 = 10^8$ (A–C) and $s_{AB} = 0.15$ and $N_0 = 10^6$ (D–F). The theoretical curves are based on File S3, Equation S3.1 (for $s_{ab} = -1$), Equation S3.10 (for $s_{ab} = -0.005$), and Equation S3.12 (for $s_{ab} = -0.99$). Symbols denote simulation results. Each simulation point is the average of 10^5 replicates.

since the rescue probability (Equation 7) is a concave function of n_{AB} (it does not help getting rescued twice). Negative fluctuations thus have a stronger effect on P_{rescue} than positive fluctuations and drift reduces the probability of evolutionary rescue. This effect is most pronounced for tight linkage, but is attenuated by recombination (*e.g.*, Figure 1D). Since recombination pulls genotype frequencies closer to linkage equilibrium, it overall dampens fluctuations in LD and along with it fluctuations in n_{AB}. For positive epistasis prior to the environmental change (Figure 1F), this results in a nonmonotonic dependence of P_{rescue} on recombination. For small r ($r \lesssim 2|\sigma|$, see Equation 8), recombination is deleterious since it breaks up the positive LD built up by epistasis; for larger r, the positive effect of recombination (attenuating drift) dominates. Figure S3.1 in File S3 disentangles the effects of epistasis and drift.

If the wild type is not lethal in the new environment (Figure 1, open symbols), recombination is deleterious after the environmental change. Note that even a slight increase of the wild-type fitness above lethality drastically influences the outcome for recombination, $r \gtrsim s_{AB}$ (Figure 1, open triangles). The presence of the wild type during the first few generations after the environmental change is sufficient to break up double mutants and to hamper their establishment probability significantly. If the wild type is quite fit (Figure 1, open circles) and recombination strong ($r \gtrsim s_{AB}$), rescue becomes impossible. This corresponds to observations in populations of constant size where the crossing of fitness valleys is prevented by strong recombination (Crow and Kimura 1965; Jain 2010; Weissman *et al.* 2010).

For a slowly decaying wild type, the results are robust to deviations of the fitness of single mutants from strict lethality; however, for a lethal wild type, chances of rescue increase considerably when single mutants have a fitness slightly larger than zero (see File S3, section S3.1 and Figure S3.2; see below for the scenario $s_{Ab} = s_{aB} = s$ and $s_{ab} = -1$).

Scheme 2: One single mutant is viable and the other is lethal

Viability of one of the single mutants, say *Ab*, opens up new pathways to rescue since new double mutants can be generated by mutation after the switch in the environment. Rescue can occur via (a) double mutants from the standing genetic variation as in the previous paragraph, (b) mutation of single mutants from the standing genetic variation after the environmental change, and—if the wild type is viable in the new environment—(c) two-step mutation after the environmental change (*i.e.*, generation of single mutants and subsequently double mutants, both by *de novo* mutation). Our aim in this section is to study the relative importance of standing variation *vs.* new mutation in two-locus rescue. In File S3, section S3.2, we treat both the *Ab* single mutants and the *AB* double mutants stochastically, using a two-type branching process, to derive the rescue probability (*cf.* Uecker *et al.* 2015). This is necessary for a quantitatively precise approximation. For a qualitative assessment of the relative importance of rescue pathways, it is sufficient to stick to a simple deterministic treatment of all single-mutant dynamics.

If the wild type either is lethal or disappears sufficiently slowly, each single mutant has approximately the same chance to generate a permanent lineage of *AB* mutants, independently of whether it is already present at the time of environmental change or arises later. To compare the relative importance of pathways b and c, it is hence sufficient to

compare the number of *Ab* mutants in the standing genetic variation $\sim(-uN_0/\sigma_{Ab})$ with the number of *Ab* mutants that get newly generated during population decline, $\sim(-uN_0/s_{ab})$ [assuming $n_{ab}\approx N_0(1+s_{ab})^t$]. Accordingly, the contribution from pathway c is larger than that from b if $s_{ab}>\sigma_{Ab}$. To compare pathways a and b, we compare the exponent of Equation 8, assuming $\sigma_{Ab}=\sigma_{aB}$, with the number of successful *AB* mutants issued from *Ab* mutants in the standing genetic variation. If we assume a deterministic decay of the *Ab* mutants in the new environment, $n_{Ab}(t)\approx\overline{n}_{Ab}(1+s_{Ab})^t$, the latter is given by $p_{\text{est}}^{(AB)}\sum_{t=0}^{\infty}un_{Ab}(t)=p_{\text{est}}^{(AB)}\left(u^2N_0/\sigma_{Ab}s_{Ab}\right)$, where $p_{\text{est}}^{(AB)}\approx 2s_{AB}$ if the wild type is lethal and $p_{\text{est}}^{(AB)}\approx 2\max[(s_{AB}-r),0]$ if the wild type disappears slowly. With $E_1=0$ or r large, this contribution is larger than rescue via pathway a if $s_{Ab}>\sigma_{Ab}$, *i.e.*, if the growth parameter of single mutants is larger in the new than in the old environment; for $r\to 0$, it is larger if $s_{Ab}>\sigma_{Ab}+E_1/2\approx(1/2)\sigma_{AB}$. Overall, we obtain for rescue via pathway b or c

$$P_{\text{rescue}}^{\textit{de-novo}}\approx 1-e^{-\frac{u^2N_0}{\sigma_{Ab}s_{Ab}}p_{\text{est}}^{(AB)}-\frac{u^2N_0}{s_{ab}s_{Ab}}p_{\text{est}}^{(AB)}}, \tag{9}$$

where the first summand in the exponent accounts for pathway b and the second summand for pathway c, using that every single mutant leaves on average $-u/s_{Ab}$ double-mutant offspring.

If the wild type is lethal (Figure 2A), the contribution of route b to rescue is independent of recombination, and recombination has an influence on rescue only via its effect on the number of double mutants in the standing genetic variation (compare Figure 2A with Figure 1D). If the wild type is viable (Figure 2B), recombination is deleterious after the environmental change (*cf*. Figure 1).

The more precise analysis in section S3.2 of File S3 extends to $s_{Ab}>0$. Then, rescue does not require the generation of the double mutant. We discuss in File S3, section S3.2, when focusing on establishment of type *Ab* is sufficient under these conditions.

Scheme 3: Both single mutants are viable in the new environment

In our third scheme, we turn to scenarios in which single mutants have absolute fitness $0<1+s_{Ab},1+s_{aB}<1$ in the new environment such that the last pathway to rescue opens up: in addition to the previous routes, the rescue mutant can now be generated by recombination after the environmental change. As in the phase prior to the environmental switch, the net role of recombination after the environmental change depends on the linkage disequilibrium among types: with negative LD, the net effect of recombination is to generate rescue mutants; with positive LD, it instead breaks them up. The expected LD, in turn, depends on the growth rates (fitnesses) of the four types: positive/negative epistasis entails positive/negative LD. With a switch in the selection regime at the time of environmental degradation, we can thus distinguish four basic scenarios, combining positive or negative epistasis before the switch with either positive or negative epistasis after the change, keeping or reversing the role of recombination.

For our analysis, we consider two cases for the fitness scheme after the environmental change: (1) negative epistasis with $s_{Ab}=s_{aB}=s$ and $s_{ab}=-1$ (*i.e.*, the wild type is lethal) and (2) positive epistasis with $s_{ab}\geq s_{Ab}=s_{aB}=s$. Epistasis in the original environment is positive or negative. For simplicity, we assume equal single-mutant fitnesses, $\sigma_{Ab}=\sigma_{aB}=\sigma$. Note that, with this choice, we have $n_{Ab}(t)=n_{aB}(t)$ for all times, as long as drift can be ignored.

Rescue from double mutants in the standing genetic variation can be evaluated as above (scheme 1) with $p_{\text{est}}^{(AB)}\approx 2s_{AB}$ for scenario 1 and $p_{\text{est}}^{(AB)}\approx 2\max[(s_{AB}-r),0]$ for scenario 2. To determine the total probability of evolutionary rescue, we need to add rescue from double mutants that originate after the environmental change. These are generated at a time-dependent rate $\left(r\frac{n_{Ab}(t)n_{aB}(t)}{N(t)}+u(n_{Ab}(t)+n_{aB}(t))\right)$. In scenario 1, with $n_{Ab}(t)=n_{aB}(t)$ and $N(t)=n_{Ab}(t)+n_{aB}(t)$ (which holds as long as the *AB* mutant is rare), this simplifies to $(r/2+2u)n_{Ab}(t)$. The rate of decline of single mutants is considerably enhanced by recombination in this scenario, since half of all recombination events occur among *Ab* and *aB* types. Each such recombination event breaks up both single mutants. The single-mutant types hence decay at rate $|s-r/2-2u|$, generating approximately $\sum_{t=0}^{\infty}\left(\frac{r}{2}+2u\right)n_{Ab}(t)=\frac{r/2+2u}{r/2-s+2u}\overline{n}_{Ab}$ double mutants on their way to extinction. Each of these double mutants establishes a permanent lineage with probability $2s_{AB}$. The combination of these two rescue pathways—generation of the rescue type by mutation or recombination after the environmental change—is hence given by

$$P_{\text{rescue}}^{\textit{de-novo}}=1-e^{-2s_{AB}\frac{r/2+2u}{r/2+2u-s}\frac{uN_0}{-\sigma}}, \tag{10}$$

which increases with r. If epistasis is negative prior to the environmental change, recombination is hence advantageous in both phases and P_{rescue} increases with r (Figure 3A). If, on the other hand, epistasis is positive in the old environment, the effect of recombination changes from negative to positive between the two phases. The negative effect in the old environment and the positive effect in the new environment act on different recombination scales: the negative effect levels off for $r\gg 2|\sigma|$ (see Equation 8). As can be seen from Equation 10, the positive effect of recombination levels off once $r\gg -s$. In Figure 3B, selection is stronger in the new environment ($|s|\gg|\sigma|$). Moreover, $P_{\text{rescue}}^{\textit{de-novo}}$ is small for weak recombination, since the single mutants decay rapidly after the environmental change. Therefore, the negative effect of recombination dominates for small r; the positive effect takes over as recombination increases.

Scenario 2, used in Figure 3, C and D, is more complex. The proportion of single mutants changes during population decline (even for $s_{ab}=s$, since new single mutants arise during

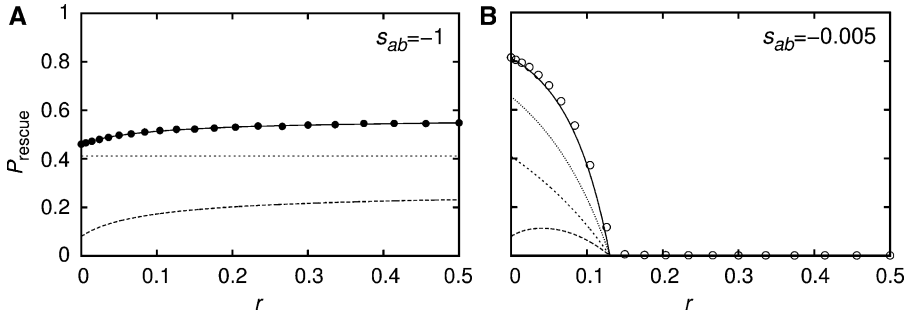

Figure 2 Probability of evolutionary rescue as a function of recombination when one single mutant is lethal and the other one viable. In A, the wild type is lethal; in B, it is only mildly deleterious. Solid line (with simulation symbols), total probability of evolutionary rescue; long-dashed line, rescue only via double mutants from the standing genetic variation; short-dashed line, rescue via single mutants from the standing genetic variation (which subsequently mutate); dotted line (only B), rescue from two-step *de novo* mutation. Parameter values: $\sigma_{Ab} = \sigma_{aB} = -0.01$, $\sigma_{AB} = -0.0199$ (*i.e.*, no epistasis before the environmental change), $s_{Ab} = -0.005$, $s_{aB} = -1$, $s_{AB} = 0.15$, $u = 10^{-5}$, $N_0 = 10^6$. The theoretical curves are based on File S3, Equation S3.14 (A) and Equation S3.20 (B). Each simulation point is the average of 10^5 replicates.

population decline). The rate at which single mutants recombine to generate double mutants is hence not constant and the approximation for the total number of double mutants that get newly generated after the environmental change does not take a simple form. However, it is still possible to calculate it analytically; see Equations S3.37 and S3.43 in File S3. Due to the presence of the wild type, the rate is much smaller than 1/2. Moreover, recombination reduces the establishment probability of the rescue mutant to $p_{\text{est}}^{(AB)} \approx 2\max[(s_{AB} - r), 0]$, rendering rescue impossible for strong recombination. If epistasis is negative prior to the environmental change such that *AB* mutants are underrepresented in the standing genetic variation without the aid of recombination, we find that $P_{\text{rescue}}(r)$ displays an intermediate maximum (Figure 3C and File S3, Figure S3.3). If epistasis is positive both in the old and in the new environment, deterministic theory predicts that recombination is always harmful. However, in finite populations with a small number of double mutants, recombination has a positive effect by attenuating the effect of drift (as described for scheme 1, Figure 1). As a consequence, P_{rescue} displays a minimum and a maximum in Figure 3D.

Scheme 4: Both single mutants have fitness greater than one

If single mutants have fitness greater than one, formation of the double mutant is not required for rescue, but can still increase the rate of rescue if the double mutant is considerably fitter than the single mutants. However, formation of the double mutant comes at the cost of losing two single mutants. Keeping the single mutants intact can therefore be better for rescue than generating the double mutant if the latter is only slightly fitter than the single mutants.

For simplicity, we consider scenario 1 from the previous section with lethal wild type after the change but allow $s_{Ab} = s_{aB} = s$ to be greater than zero. Under these conditions, recombination cannot break the double mutant after the change in the environment. The role of recombination simply is to convert the different rescue types into each other, more precisely to turn two single mutants (that are now also rescue genotypes) into one double mutant. One individual of type *AB* establishes a permanent lineage with probability $\approx 2s_{AB}$ while one individual of type *Ab* (or *aB*) establishes a permanent lineage of single mutants with probability $2s$. Intuitively, conversion of two single mutants into one double mutant therefore pays off if $s_{AB} \gg 2s$ and is deleterious for rescue if $s_{AB} \ll 2s$. Recombination hence increases the rate of rescue if $s_{AB} \gg 2s$ and decreases the rate of rescue if $s_{AB} \ll 2s$; it has little effect if $s_{AB} \approx 2s$ (see Figure 4). We formalize this argument in File S3, section S3.4.

Notable observations

To conclude, we point out two effects of recombination on rescue probabilities that might contradict spontaneous intuition. First, with recombination, a high frequency of wild-type individuals after the environmental change is a potent force to inhibit rescue by double mutants. Consequently, a slower decay of the wild-type population often reduces, rather than promotes, the chances for population survival. While a slower decay leads to a higher rate of new single mutants, the latter are less likely to meet and recombine in the presence of a dominating wild type. Also, if a double mutant is generated, it is likely to be broken up by recombination. Depending on the strength of recombination, the rate of rescue decreases or increases or displays an intermediate minimum as a function of the wild-type fitness (see Figure 5A; *cf*. also File S3, Figure S3.3). Indeed, we obtain a clear decrease in P_{rescue} for almost the entire range of wild-type fitness s_{ab}, unless recombination is extremely weak. Only for very high wild-type fitness, approaching viability ($s_{ab} = 0$), P_{rescue} steeply increases again (*cf*. Figure 5A). Note that the transition from recombination being beneficial to being harmful occurs for $E_1 > 0$; this is likely due to the concavity of P_{rescue} in combination with stochasticity, similarly to the effect of drift on rescue from the standing genetic variation.

Second, with recombination, single-mutant types can act as an important buffer to environmental change, even if they are not able to rescue a population on their own. As a consequence, two-step rescue does not need to be less likely than single-step rescue with a single locus and direct generation of the rescue type from the wild type by a single mutation (see Figure 5B). Imagine a situation where the wild type is lethal in the new environment. Single-step rescue now relies entirely on the rescue type individuals that are present at the

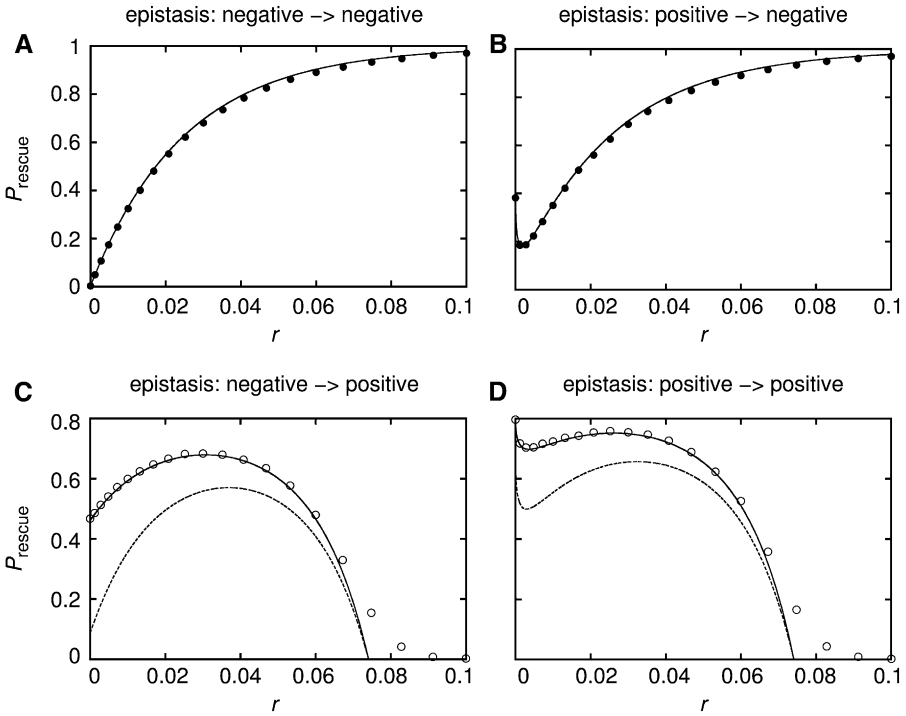

Figure 3 Probability of evolutionary rescue as a function of recombination for different patterns of epistasis before and after the environmental change. Solid line, total probability of rescue; dashed line, probability of rescue from the standing genetic variation (*i.e.*, without new mutations after the environmental change). In A and B, both lines coincide. The analytical predictions in A and B are based on File S3, Equation S3.32 and its components; the analytical predictions in C and D are based on File S3, Equation S3.38 and its components. Parameter values: $N_0 = 10^8$, $u = 2 \times 10^{-6}$, and $\sigma_{Ab} = \sigma_{aB} = -0.01$; first row (A and B), $s_{Ab} = s_{aB} = -0.5$, $s_{ab} = -1$, $s_{AB} = 0.002$, and $\sigma_{AB} = -0.1$ (*i.e.*, $E_1 \approx -0.08$, A), $\sigma_{AB} = -0.0001$ (*i.e.*, $E_1 \approx 0.02$, B); second row (C and D), $s_{ab} = s_{Ab} = s_{aB} = -0.03$, $s_{AB} = 0.08$, and $\sigma_{AB} = -0.1$ (*i.e.*, $E_1 \approx -0.08$, C), $\sigma_{AB} = -0.0001$ (*i.e.*, $E_1 \approx 0.02$, D). Symbols denote simulation results. Each simulation point is the average of 10^5 replicates.

time of environmental change. In two-step rescue, single mutants might still be present in the new environment and can generate the rescue mutant by mutation or recombination at a high rate. Even the number of double mutants in the standing genetic variation can be higher for two-step than for single-step rescue if the mutation rate is high or epistasis strongly positive (see File S3, section S3.5 for more details).

Discussion

Following severe environmental change, populations might find themselves maladapted to the new conditions, and a race between population decline and adaptive evolution begins. In conservation biology, the desired outcome of this race is persistence of the population; in medicine, in contrast, one aims at eradication of the pathogen from the human body. Adaptation to the new environmental conditions is often contingent on allelic changes at more than one locus. This holds, in particular, for resistance to multiple drugs in combination drug therapy or pesticide mixtures in agriculture. Complex rescue, requiring adaptation at multiple loci, is expected to lead to severely reduced probabilities of rescue (or resistance). However, the prediction of these probabilities can be surprisingly complicated if there is recombination among the target loci.

In this article, we have analyzed a generic two-locus model to clarify the role of recombination in evolutionary rescue. We find that depending on the fitness scheme of mutations, recombination can make two-step rescue even more likely to happen than single-step rescue but it can also prevent rescue entirely (see Figure 3, C and D, and Figure 5B). Recombination acts to reduce positive or negative LD that is built up by epistasis and it weakens fluctuations in LD caused by genetic drift. Since there are two phases of selection—before and after the environmental change—and drift, recombination acts threefold, where the effects can go in different directions (increasing or decreasing the rate of rescue) and show at different scales. As a consequence, dependence of rescue on recombination can be nonmonotonic with multiple ups and downs (see, *e.g.*, Figure 3D). Also the dependence of rescue on the wild-type dynamics is nontrivial, with a slow decline of the wild type not always being better for population survival than a fast eradication (see Figure 5A).

The role of epistasis

As is well known from classical population genetics, whether recombination speeds up or slows down adaptation strongly depends on the sign of epistasis (Felsenstein 1965). In scenarios of evolutionary rescue, there are two phases of selection with potentially epistatic interactions between loci, before and after the change in the environment, and the role of recombination is affected by epistasis in both phases. Experimentally, the strength and sign of epistasis have been measured across a variety of systems (Bonhoeffer *et al.* 2004; Kouyos *et al.* 2007; Trindade *et al.* 2009; Silva *et al.* 2011), reporting all forms of epistasis. Moreover, several studies have investigated the influence of the environment on the epistatic interactions between mutations, finding that both the strength and the sign of epistasis can change as the environment changes (Remold and Lenski 2004; Lalic and Elena 2012; De Vos *et al.* 2013; Flynn *et al.* 2013). This shows that for a comprehensive picture of the role of epistasis on adaptation upon environmental change, all possible fitness schemes in both environments need to be studied.

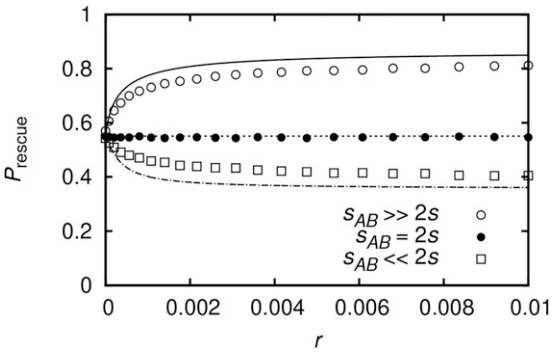

Figure 4 Probability of evolutionary rescue as a function of recombination. For $s_{AB} \gg 2s$, P_{rescue} increases with recombination; for $s_{AB} = 2s$, recombination has no effect on rescue; for $s_{AB} \ll 2s$, P_{rescue} decreases with recombination. Parameter values: $N_0 = 10^7$, $u = 2 \times 10^{-6}$, $\sigma_{Ab} = \sigma_{aB} = -0.01$, $\sigma_{AB} = -0.0199$, $s_{ab} = -1$, and $s_{Ab} = s_{aB} = 10^{-4}$; open circles, $s_{AB} = 5 \times 10^{-4} \gg 2s$; solid circles, $s_{AB} = 2 \times 10^{-4} = 2s$; and squares, $s_{AB} = 0.11 \times 10^{-4} \ll 2s$. The theoretical predictions are based on File S3, Equation S3.45 combined with Equation S3.1. Symbols denote simulation results. For the simulations, we consider the population as rescued if the total number of mutant genotypes has reached 20% carrying capacity. Each simulation point is the average of 10^5 replicates.

Epistasis leads to linkage disequilibrium, which is broken up by recombination. Recombination thus counteracts selection. The scale at which recombination is effective is determined by the strength of epistatic selection. Since the strength of selection can be different before and after the environmental change, the relevant recombination scales can be different, too, and if the sign of epistasis changes with the environment, the probability of evolutionary rescue depends nonmonotonically on recombination.

We can compare our results with classical models for the crossing of fitness valleys in populations of constant size. In these models, a small but nonzero recombination rate minimizes the time to get from one fitness peak to another, while strong recombination hampers or even prevents the crossing of valleys in large populations (Jain 2010; Weissman *et al.* 2010; Altland *et al.* 2011). Epistasis is positive in this scenario. However, since double mutants are initially absent, LD is negative during a first transient phase. As the frequency of double mutants increases (supported by recombination), LD turns positive and recombination counteracts any further increase of double mutants. The valley-crossing scenario thus compares to a rescue situation with negative epistasis in the old and positive epistasis in the new environment. Indeed, we obtain analogous results under these conditions (see Figure 3C).

The role of drift

We find that genetic drift has a strong influence on rescue probabilities, even in very large populations (see, *e.g.*, File S2, Figure S2.1 with $N_0 = 10^8$). This may seem surprising, but can be understood because the decisive quantity for rescue is the number of double mutants, which is potentially small even if the total population size is large. Importantly, stochastic fluctuations in the number of rescue types not only entail a stochastic outcome (extinction or survival), but also have a directional (negative) effect on the rescue probability. This is because for any given population, the probability of evolutionary rescue is a concave function of the number of rescue mutants in the standing genetic variation or generated *de novo*. Consequently, the decrease in the rescue probability due to negative fluctuations in the number of rescue types is larger than the corresponding increase due to positive fluctuations. This effect is strong if the number of double mutants is small and their establishment probability large.

The effect is most prominent for two-step adaptation at a single locus, *i.e.*, in the absence of recombination. Previous theory for two-step rescue for that case has described all genotype frequencies in the standing genetic variation deterministically (Iwasa *et al.* 2003, 2004). While this is appropriate when the number of double mutants is large and their establishment probability small, the approach strongly overestimates the probability of evolutionary rescue if these conditions are not met (see File S2 and Figure S2.1 in File S2).

Recombination attenuates this effect of drift by pulling the number of double mutants closer to its expected value and thus increases the probability of rescue. We find that the decrease of fluctuations in LD (and in the number of double mutants) affects rescue equally or sometimes even more strongly than the reduction of directional LD (increasing or decreasing the mean number of double mutants) that has been built up by epistasis. We finally note that the interaction of drift and recombination described here is different from the effect of recombination in the presence of Hill–Robertson interference in finite populations that has been described previously (Barton and Otto 2005; Roze 2014). This latter mechanism works through a small bias toward negative LD on average because selection acts asymmetrically on symmetric fluctuations in LD. This is negligible in our model, while fluctuations in LD turn out to be very prominent.

The population dynamics

As long as the rescue type is rare, the population dynamics are shaped by the dynamics of the wild type and the single mutants. Dependence of rescue on the dynamics of single mutants is as expected: the slower the decay, the higher the chance of rescue. The dependence on the dynamics of the wild-type population size is more complex and shaped by two opposing effects. By mutation of wild-type individuals, single mutants arise, which can subsequently mutate or recombine to generate the rescue type. On the other hand, recombination with wild-type individuals breaks up the rescue type. Presence of the wild type hence increases the rate at which the rescue mutant is generated but decreases its establishment probability. As a consequence, dependence of rescue on the rate of decline of the wild-type population is nontrivial. We find that the rate of rescue decreases with wild-type fitness over a large parameter range but it can also increase and be overall nonmonotonic as a function of wild-type fitness (see Figure 5A).

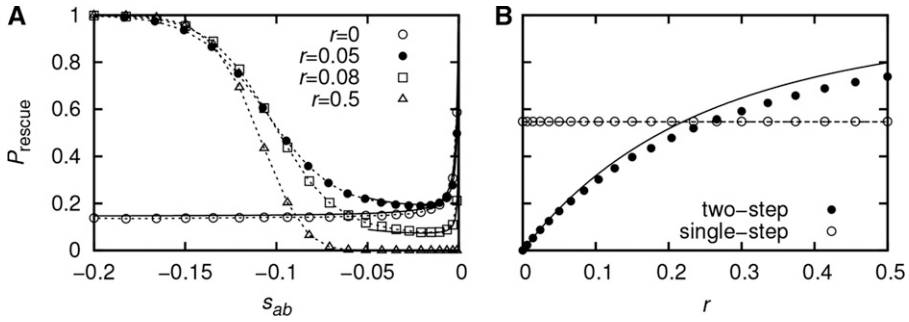

Figure 5 Probability of evolutionary rescue. (A) Probability of evolutionary rescue as a function of wild-type fitness for various values of r. Solid curves constitute analytical predictions and are based on File S2, Equation S2.2 ($r = 0$) and File S3, Equation S3.38 with Equation S3.43 ($r > 0$). Dotted lines connect simulation points and are included to guide the eye. Parameter values: $\sigma_{Ab} = \sigma_{aB} = -0.01$, $\sigma_{AB} = -0.0199$, $s_{Ab} = s_{aB} = -0.05$, $u = 10^{-5}$, $N_0 = 10^6$, and $s_{AB} = 0.1$. (B) Probability of evolutionary rescue when the rescue mutant is one or two mutational steps away. The analytical predictions are based on File S3, Equation S3.32 (for two-step rescue) and Equation S3.49 (for single-step rescue). Parameter values: $u = 2 \times 10^{-6}$, $N_0 = 10^7$, $\sigma_{Ab} = \sigma_{aB} = -0.01$, $\sigma_{AB} = -0.1$, $s_{ab} = -1$, $s_{Ab} = s_{aB} = -0.5$, and $s_{AB} = 0.002$. Symbols denote simulation results. For A, we consider a population as rescued when the number of double mutants has reached 20% carrying capacity (increasing the threshold to 30% did not change the results). Each simulation point is the average of 10^5 replicates.

For strong recombination, a high frequency of wild-type individuals prevents rescue entirely. Importantly, dependence of rescue on the presence or absence of the wild type can be very sensitive such that even a slight increase of the wild-type fitness above lethality can significantly reduce the probability of population survival (see Figure 1).

Violations of the simple rule for drug therapy to hit hard (the faster the wild-type population disappears, the lower the risk of resistance) have been found before as a consequence of competitive release: if the fitness of the rescue type is density dependent, a fast eradication of the wild type enhances rescue by freeing up resources (Torella *et al.* 2010; Read *et al.* 2011; Peña-Miller *et al.* 2013; Uecker *et al.* 2014). Note that here we find that the rule is violated also in a model without competition for resources and with density-independent fitness. Our results imply that to prevent resistance, it is of vital importance to suppress the single mutants efficiently while it can be preferable to remove the wild type slowly. Otherwise the single mutants can act as a reservoir for mutations from which the rescue type can be generated even if the single mutants are not long-term viable themselves and even if they have a very low fitness (see File S3, Figure S3.2). As a consequence, two-step rescue can be even more likely than single-step rescue (see Figure 5B).

Normally, we expect that in the presence of several drugs, a mutant that is resistant to one of the drugs has a higher fitness than the wild-type strain. For example, Chait *et al.* (2007) find this behavior when they expose wild-type and doxycycline-resistant *Escherichia coli* bacteria to a drug combination of doxycycline and erythomycin. The two drugs act synergistically, *i.e.*, the wild type has a lower fitness in the presence of both drugs than expected from the single-drug effects. In a combination of doxycycline and ciprofloxacin, however, Chait *et al.* (2007) show that the doxycycline-resistant mutant is even less fit than the wild type at certain drug concentrations [we apply such a fitness scheme in the limit of a lethal single mutant(s) in Figure 1 and in Figure 2B]. At these concentrations, the two drugs display "suppression interaction"; *i.e.*, the wildtype has a higher fitness in the presence of both drugs than in the presence of just one drug, which is an extreme form of antagonistic drug interactions (one drug attenuates the effect of the other). Based on these findings, Torella *et al.* (2010) developed a mathematical model for the evolution of multidrug resistance under synergistic and antagonistic drug interactions (implementing no form of recombination). The model shows that resistance evolves less easily under antagonistic interactions but again only if competition among cells is high (for experiments, see Hegreness *et al.* 2008). Our results suggest that even without competition, antagonistic drug interactions (with a relatively fit wild type but unfit single mutants) can strongly hamper the evolution of resistance for infections with pathogens that readily recombine *in vivo*, such as HIV.

Likewise, our finding that two-step rescue can be more likely than single-step rescue can be important when assessing the risk of drug resistance in combination-drug *vs.* single-drug therapy. To avoid excessive toxicity, each single drug might need to be given in a lower dose in combination drug treatment. While the wild type, which is sensitive to all drugs, gets successfully eliminated, mutants that are resistant to one of the drugs might then not get fully suppressed by the second drug. A situation similar to that shown in Figure 5B arises; for high recombination between the loci conferring resistance to either drug, the risk of drug resistance is higher in the drug combination scheme than if a single drug were applied at a high dose.

Limitations and extensions

Our analysis gives a comprehensive overview of the role of recombination in the two-locus model for evolutionary rescue. However, quantitatively accurate analytical results are possible only in parts of the parameter range. Most importantly, if both single-mutant types are viable and can recombine, we need to describe their frequencies deterministically. This requires a sufficiently large population size. We illustrate the limits of this approach in File S4.

Our model describes the most basic situation both on the genetic and on the ecological side (two loci, two alleles per locus, panmictic population, and sudden environmental shift). On the genetic side, the incorporation of more loci

and the consideration of more complex fitness landscapes constitute a logical next step. On the ecological side, a variety of extensions would help to gain a more comprehensive understanding of two-step rescue with recombination. A gradual instead of sudden deterioration of the environment influences the population dynamics, which, as discussed above, play a relevant role in rescue. Likewise, population structure with parts of the habitat deteriorating later than others changes the rate of disappearance of the wild type (Uecker *et al.* 2014).

Our "minimal model" approach means, of course, that the results cannot be directly applied to concrete cases of resistance evolution. While we expect that the basic principles observed in this study should hold under general conditions, further factors need to be included for specific cases. For example, recombination in HIV is density dependent since multiple infection of a cell is required for recombination to occur. Also, phenotypic mixing does not allow for a simple correspondence between phenotype and genotype and long-lived cells lead to specific population dynamics. Likewise, all three forms of bacterial recombination—conjugation, transduction, and transformation—differ significantly from the simple recombination scheme applied in this study, requiring two mating types, the action of bacteriophages or the release and uptake of DNA molecules into/from the environment.

We focused entirely on the probability of evolutionary rescue, leaving other aspects of rescue unexplored. It would be interesting to find out how recombination affects the time to rescue and the shape of population decline and recovery given that rescue occurs (*cf*. Orr and Unckless 2014 for a study on these aspects in a one-locus model). The minimum population size of the U-shaped rescue curve is predictive for the amount of standing genetic variation that is preserved over the course of adaptation. The latter in turn affects how well a population can respond to subsequent environmental change.

To summarize, we have analyzed a generic model of two-step rescue with recombination. We find that the role of recombination in rescue is complex and ambivalent, ranging from highly beneficial to highly detrimental. Since recombination of rescue mutants with wild-type individuals destroys the rescue type, a fast eradication of the wild type can counterintuitively promote rescue even in the absence of competition for resources. A high fitness of single mutants always fosters rescue even if they cannot persist in the long-term in the environment themselves. Recombination of single mutants that provide a buffer to environmental change can render two-step rescue even more likely than one-step rescue.

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The Role of Recombination in Evolutionary Rescue

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Supporting information: The role of recombination in evolutionary rescue

File S1: General notes on the analysis

Major parts of the analysis are based on branching process approximations. We model the number of double mutants (and occasionally also the number of single mutants) in the standing genetic variation by a subcritical branching process with immigration, where "immigration" happens through mutation or recombination. For the establishment probability of a type AB individual, we apply results from the theory of time-homogeneous or time-inhomogeneous single-type branching processes. In order to determine the probability that a type Ab individual gives rise to a permanent lineage of AB individuals by mutation, we use a two-type branching process. Although the model is formulated in discrete time, we resort to branching processes in continuous time for the mathematical analysis. In the following, we first state some general mathematical results from branching process theory. We thereafter apply them to derive some building blocks that we use repeatedly in the subsequent analysis in File S2 and File S3.

S1.1 Mathematical results from branching process theory

Probability generating function for the number of individuals in a subcritical single-type branching process with immigration. Following Sewastjanow (1974, p. 163), we can calculate the probability generating function (p.g.f.) for the number of individuals in a subcritical branching process with immigration. Individuals reproduce at rate λ and die at rate μ. Immigration happens at rate m. We define the two infinitesimal generating functions

$$f(y) = \mu - (\lambda + \mu)y + \lambda y^2, \tag{S1.1a}$$
$$g(y) = -m + my. \tag{S1.1b}$$

Let P_k be the probability to have k individuals in the limit $t \to \infty$ and

$$F(y) = \sum_{k=0}^{\infty} P_k y^k \tag{S1.2}$$

It then holds

$$\begin{aligned} F(y) &= \exp\left[\int_y^1 \frac{g(x)}{f(x)}\mathrm{d}x\right] \\ &= \left(\frac{\lambda-\mu}{y\lambda-\mu}\right)^{\frac{m}{\lambda}}. \end{aligned} \tag{S1.3}$$

For $\lambda = \frac{1}{2} + \frac{\sigma}{2}$ and $\mu = \frac{1}{2} - \frac{\sigma}{2}$, this gives

$$F(y) = \left(\frac{2\sigma}{y + y\sigma + \sigma - 1}\right)^{\frac{2m}{1+\sigma}}. \tag{S1.4}$$

From the p.g.f., the stationary distribution of the number of individuals can be obtained as

$$P_k = \frac{1}{k!}\frac{\mathrm{d}}{\mathrm{d}y}F(y)|_{y=0} = \frac{1}{k!}\left(\frac{2\sigma}{\sigma-1}\right)^{\frac{2m}{\sigma+1}+k} \cdot \prod_{i=1}^{k} \frac{2m + (i-1)(1+\sigma)}{(-2\sigma)} \tag{S1.5}$$

for $k > 0$ and $P_0 = F(0)$.

Establishment probability of a reducible two-type branching process. Consider a branching process with two types. Type i reproduces at rate λ_i and dies at rate μ_i. Type 2 turns into type 1 at rate u_{eff}.

The survival probability of a process founded by one individual of type 1 is given by (Allen, 2011, p. 253)

$$p_{\mathrm{est}}^{(1)} = \begin{cases} \frac{\lambda_1-\mu_1}{\lambda_1} & \text{if} \quad \lambda_1 > \mu_1, \\ 0 & \text{else.} \end{cases} \tag{S1.6}$$

The establishment probability of a process founded by a single individual of type 2 can be obtained by solving the equation

$$1 - p_{\mathrm{est}}^{(2)} = \frac{\mu_2}{\lambda_2+\mu_2+u_{\mathrm{eff}}} + \frac{u_{\mathrm{eff}}}{\lambda_2+\mu_2+u_{\mathrm{eff}}}(1-p_{\mathrm{est}}^{(1)}) + \frac{\lambda_2}{\lambda_2+\mu_2+u_{\mathrm{eff}}}(1-p_{\mathrm{est}}^{(2)})^2, \tag{S1.7}$$

where the smaller root has to be taken (Uecker *et al.*, 2015):

$$\begin{aligned} p_{\mathrm{est}}^{(2)} &= 1 - \frac{\lambda_2+\mu_2+u_{\mathrm{eff}} - \sqrt{(\lambda_2+\mu_2+u_{\mathrm{eff}})^2 - 4(u_{\mathrm{eff}}(1-p_{\mathrm{est}}^{(1)})+\mu_2)\lambda_2}}{2\lambda_2} \\ &= 1 - \frac{\lambda_2+\mu_2+u_{\mathrm{eff}} - \sqrt{(\lambda_2-\mu_2-u_{\mathrm{eff}})^2 + 4\lambda_2 u_{\mathrm{eff}} p_{\mathrm{est}}^{(1)}}}{2\lambda_2}. \end{aligned} \tag{S1.8}$$

With $\lambda_2 = \frac{1}{2} + \frac{s}{2}$ and $\mu_2 = \frac{1}{2} - \frac{s}{2}$, this yields:

$$p_{\mathrm{est}}^{(2)} = 1 - \frac{1 + u_{\mathrm{eff}} - \sqrt{(s-u_{\mathrm{eff}})^2 + 2(1+s)u_{\mathrm{eff}}p_{\mathrm{est}}^{(1)}}}{1+s}. \tag{S1.9}$$

Establishment probability of an inhomogeneous single-type branching process. The establishment probability of a single allele with time-dependent birth rate $\lambda(t)$, death rate $\mu(t)$, and growth parameter $\lambda(t) - \mu(t) = s_{\text{eff}}(t)$ that arises at time T in a population is given by (Kendall, 1948; Uecker and Hermisson, 2011)

$$p_{\text{est}}(T) = \frac{2}{1 + \int_T^\infty (\lambda(t) + \mu(t)) e^{-\int_T^t s_{\text{eff}}(\tau) \mathrm{d}\tau} \mathrm{d}t}. \tag{S1.10}$$

The extinction time of a single-type branching process. Consider a subcritical branching process with an initial number of n_0 individuals. Individuals reproduce at rate λ and die at rate μ. From the probability that the process has gone extinct by time t, $P_0(n_0, t)$, (see Uecker and Hermisson, 2011), we immediately obtain the distribution of the extinction time T_{ext}:

$$P(T_{\text{ext}} \leq t) = P_0(n_0, t) = \left(\frac{\mu(1 - e^{-(\lambda-\mu)t})}{\lambda - \mu + \mu(1 - e^{-(\lambda-\mu)t})} \right)^{n_0}. \tag{S1.11}$$

We denote by

$$p^{(\text{ext})}(t) = \frac{\mathrm{d}}{\mathrm{d}t} P(T_{\text{ext}} \leq t) \tag{S1.12}$$

the corresponding probability density.

S1.2 Essential building blocks

In order to match the results from the continuous-time approximation to the discrete-time model, we need to make sure that the growth behavior and the amount of drift are the same (Uecker *et al.*, 2014). First, in order to guarantee that the long-term growth behavior is the same, we replace the growth parameter σ from the discrete-time model by $\ln(1 + \sigma)$ in the continuous-time approximation whenever long-term growth is essential. In order to generate the same amount of drift, birth and death rates of individuals must sum up to 1 (at least in the diffusion limit). In a model with selection, this can be achieved in various ways, by distributing the effect of the effective growth parameter σ (or $\ln(1 + \sigma)$) on the birth and death rates. If not stated otherwise, we usually do this symmetrically, i.e., $\lambda = \frac{1}{2} + \frac{\sigma}{2}$ and its death rate as $\mu = \frac{1}{2} - \frac{\sigma}{2}$. This is appropriate as long as selection is not too strong. For very large (positive or negative) σ, one of the rates can turn negative. In that case, we switch to a different parametrization (and explicitly state this).

Throughout the analysis, we ignore back mutation. We furthermore assume that the mutation rate is small enough that we can neglect direct generation of the double mutant from the wildtype.

The number of single mutants in the standing genetic variation. We assume that mutants are rare in relative frequency in the population, i.e., they only interact with wildtype individuals. This has several implications: (1) birth and death rates are constant (since mean fitness is ≈ 1), (2) a constant influx of new mutations (since $n_{ab} \approx N_0$), (3) recombination has no effect on single mutants (since mutants only recombine with wildtype individuals), (4) interactions with double mutants can be ignored.

Then, from Eq. (S1.4) with $m = uN_0(1+\sigma_{Ab})$ and $\lambda = \frac{1}{2} + \frac{\sigma_{Ab}}{2}$ and $\mu = \frac{1}{2} - \frac{\sigma_{Ab}}{2}$, we obtain the probability generating function F_{Ab} for the number of Ab mutants in the population; analogous, we obtain F_{aB}:

$$F_{Ab}(y) = \left(\frac{2\sigma_{Ab}}{y + y\sigma_{Ab} + \sigma_{Ab} - 1}\right)^{2uN_0}, \tag{S1.13a}$$

$$F_{aB}(y) = \left(\frac{2\sigma_{aB}}{y + y\sigma_{aB} + \sigma_{aB} - 1}\right)^{2uN_0}. \tag{S1.13b}$$

The mean number of Ab and aB mutants is given by

$$\bar{n}_{Ab} = \langle n_{Ab} \rangle = F'_{Ab}(1) = -\frac{uN_0}{\sigma_{Ab}}(1+\sigma_{Ab}), \tag{S1.14a}$$

$$\bar{n}_{aB} = \langle n_{aB} \rangle = F'_{aB}(1) = -\frac{uN_0}{\sigma_{aB}}(1+\sigma_{aB}). \tag{S1.14b}$$

The number of double mutants in the standing genetic variation. In a large population, in which single mutants are frequent in absolute but rare in relative numbers, their number can be well approximated by their mean value as given by Eq. (S1.14).

However, the number of double mutants is subject to strong stochasticity. Before the time of environmental change, their distribution can be modeled by a subcritical branching process with immigration. Immigration happens at rate

$$m_{AB} = \left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}\right)(1+\sigma_{AB}) + u(\bar{n}_{Ab} + \bar{n}_{aB})(1+\sigma_{AB})(1-r). \tag{S1.15}$$

As the effective selection coefficient of AB individuals, we use

$$\sigma_{AB}^{\text{eff}} = (1+\sigma_{AB})(1-r) - 1. \tag{S1.16}$$

Individuals of type AB reproduce at rate $\frac{1}{2} + \frac{1}{2}\sigma_{AB}^{\text{eff}}$ and die at rate $\frac{1}{2} - \frac{1}{2}\sigma_{AB}^{\text{eff}}$.

With Eq. (S1.4), we obtain the probability generating function $F_{AB}(s)$ for the number of double mutants in the standing genetic variation:

$$F_{AB}(y) = \left(\frac{2\sigma_{AB}^{\text{eff}}}{y + y\sigma_{AB}^{\text{eff}} + \sigma_{AB}^{\text{eff}} - 1}\right)^{\frac{2m_{AB}}{1+\sigma_{AB}^{\text{eff}}}}. \tag{S1.17}$$

The mean number of double mutants is given by

$$\begin{aligned}\langle n_{AB}\rangle = F'_{AB}(1) &= -\frac{m_{AB}}{\sigma_{AB}^{\text{eff}}} \\ &= -\frac{u^2 N_0}{\sigma_{Ab}\sigma_{aB}}(1+\sigma_{AB})\frac{r(1+\sigma_{Ab}+\sigma_{aB}+\sigma_{Ab}\sigma_{aB})-(1-r)(\sigma_{Ab}+\sigma_{aB}+2\sigma_{Ab}\sigma_{aB})}{\sigma_{AB}-r(1+\sigma_{AB})} \\ &= -\frac{u^2 N_0}{\sigma^2}(1+\sigma_{AB})(1+\sigma)\frac{r(1+\sigma)-2\sigma(1-r)}{\sigma_{AB}-r(1+\sigma_{AB})},\end{aligned} \tag{S1.18}$$

where the last line holds for $\sigma_{Ab} = \sigma_{aB} = \sigma$.

With $\sigma_{AB} = E_1 + (\sigma_{Ab} + \sigma_{aB} + \sigma_{Ab}\sigma_{aB}) = E_1 + \sigma(2+\sigma)$ and $|\sigma_{Ab}|$, $|\sigma_{aB}|$, and $|\sigma_{AB}|$ small, we can further approximate:

$$\begin{aligned}\langle n_{AB}\rangle &\approx \frac{u^2 N_0}{\sigma_{Ab}\sigma_{aB}}\frac{r-(\sigma_{Ab}+\sigma_{aB})}{r-E_1-(\sigma_{Ab}+\sigma_{aB})} \\ &= \frac{u^2 N_0}{\sigma^2}\frac{r-2\sigma}{r-E_1-2\sigma}.\end{aligned} \tag{S1.19}$$

We see that for $E_1 = 0$ (no epistasis), $\langle n_{AB}\rangle$ is independent of r; for $E_1 < 0$ (negative epistasis), $\langle n_{AB}\rangle$ increases with r; for $E_1 > 0$ (positive epistasis), $\langle n_{AB}\rangle$ decreases with r. For $r = 0$, the mean number of double mutants is given by $\frac{u^2 N_0}{\sigma^2}\frac{2\sigma}{E_1+2\sigma}$, hence strongly dependent on the degree of epistasis. For $r \gg |\sigma_{Ab} + \sigma_{aB}|$ and $r \gg |\sigma_{AB}|$, it converges to $\frac{u^2 N_0}{\sigma_{Ab}\sigma_{aB}}$, independently of epistasis.

Establishment probabilities in the absence of the wildtype. In the absence of the wildtype, the double mutant is (effectively) not broken up by recombination. With Eq. (S1.6) and $\lambda_1 = \frac{1}{2} + \frac{1}{2}\ln(1+s_{AB})$ and $\mu_1 = \frac{1}{2} - \frac{1}{2}\ln(1+s_{AB})$ (assuming $\ln(1+s_{AB}) \leq 1$, which is always the case in our examples), we obtain for the survival probability of a process which is founded by a single individual of type AB:

$$p_{\text{est}}^{(AB)} = \frac{2\ln(1+s_{AB})}{1+\ln(1+s_{AB})} \approx 2s_{AB}, \tag{S1.20}$$

where the approximation holds for s_{AB} small.

We also derive an approximation for the survival probability of a process founded by one individual of type Ab (or aB), when type AB can only be generated by mutation (either because $r = 0$ or because the other single mutant type is absent). The problem can then be assessed by means of a two-type branching process. Type Ab has birth rate $\frac{1}{2} + \frac{\hat{s}_{Ab}}{2}$ and death rate $\frac{1}{2} - \frac{\hat{s}_{Ab}}{2}$ with $\hat{s}_{Ab} = \ln(1+s_{Ab})$ (assuming $-1 \leq \ln(1+s_{Ab}) \leq 1$, which is again always fulfilled in our

examples). It turns into type AB at rate $u(1+s_{AB})$ (analogously for type aB). With (S1.9) and $Q_1 = 1 - p_{\text{est}}^{(AB)}$, we obtain the establishment probability:

$$\begin{aligned} p_{\text{est}}^{(Ab)} &= 1 - \frac{1+u(1+s_{AB}) - \sqrt{(\hat{s}_{Ab} - u(1+s_{AB}))^2 + 2u(1+s_{AB})(1+\hat{s}_{Ab})p_{\text{est}}^{(AB)}}}{1+\hat{s}_{Ab}} \\ &\approx 1 - \frac{1+u-\sqrt{(s_{Ab}-u)^2 + 4us_{AB}(1+s_{Ab})}}{1+s_{Ab}} \\ &\approx 1 - (1+u-s_{Ab} - \sqrt{(s_{Ab}-u)^2 + 4us_{AB}}) \\ &= s_{Ab} - u + \sqrt{(s_{Ab}-u)^2 + 4us_{AB}} \\ &\approx \frac{2us_{AB}}{-s_{Ab}}. \end{aligned} \tag{S1.21}$$

The last approximation holds for $s_{Ab} < 0$ and $s_{Ab}^2 \gg us_{AB}$. It can be easily interpreted: $\frac{1}{-s_{Ab}}$ is the mean number of descendants of a single Ab individual. Each of these descendants mutates with probability u, leading to a permanently establishing lineage of AB individuals with probability $2s_{AB}$.

Establishment probabilities in the presence of the wildtype. If the wildtype dominates over the single mutants at all times, the double mutant virtually always recombines with the wildtype (until it becomes frequent and rescue has occurred). Under these conditions, the effective growth parameter of the rescue type can be approximated as

$$s_{\text{eff}}(t) = \begin{cases} (1+s_{AB})(1-r) - 1 & \text{as long as the wildtype exists,} \\ s_{AB} & \text{as soon as the wildype has died out.} \end{cases} \tag{S1.22}$$

If the wildtype decays very slowly and if we can furthermore assume that no double mutants get generated once the wildtype has gone extinct, this yields for the establishment probability of the double mutant:

$$\begin{aligned} p_{\text{est}}^{(AB)} &= \begin{cases} \frac{2\ln[(1+s_{AB})(1-r)]}{1+\ln[(1+s_{AB})(1-r)]} & \text{if} \quad \ln[(1+s_{AB})(1-r)] > 0, \\ 0 & \text{else.} \end{cases} \\ &\approx 2\max[(s_{AB}-r), 0]. \end{aligned} \tag{S1.23}$$

Following the same derivation as in Eq. (S1.21), the probability that a single Ab individual will eventually give rise to a successful lineage of AB individuals is

$$\begin{aligned} p_{\text{est}}^{(Ab)} &= 1 - \frac{1+u(1+s_{AB})(1-r) - \sqrt{(\hat{s}_{Ab} - u(1+s_{AB})(1-r))^2 + 2u(1+s_{AB})(1-r)(1+\hat{s}_{Ab})p_{\text{est}}^{(AB)}}}{1+\hat{s}_{Ab}} \\ &\approx s_{Ab} - u(1-r) + \sqrt{(s_{Ab}-u(1-r))^2 + 4u(1-r)\max[2(s_{AB}-r), 0]} \\ &\approx \frac{2u(1-r)\max[(s_{AB}-r), 0]}{-s_{Ab}}. \end{aligned} \tag{S1.24}$$

The simple approximation $p_{\text{est}}^{(AB)}$, Eq. (S1.23), fails when the wildtype population size decays quickly. In case of a fast (but not instantaneous) eradication of the wildtype, we need to apply a more refined approximation for the establishment probability of type AB. The extinction time of the wildtype is a stochastic variable. If we ignore mutation and recombination, the dynamics of the wildtype is given by a subcritical branching process with initial size $n_{ab}(0) \approx N_0$, and we can calculate the distribution of the extinction time T_{ext} with the help of Eq. (S1.11). Since $\ln(1+s_{ab})$ is considerably smaller than -1 if s_{ab} is strongly negative, we deviate from our default approximation for λ and μ here and choose $\lambda = 1/2$ and $\mu = 1/2 - \ln(1+s_{ab})$ to keep selection at the right level and avoid negative birth rates. With this, we obtain

$$P(T_{\text{ext}} \leq t) = \left(\frac{1 - e^{-s_{ab}t}}{\frac{2s_{ab}}{1-s_{ab}} + 1 - e^{-s_{ab}t}} \right)^{N_0} \tag{S1.25}$$

and from this the probability density $p^{(\text{ext})}(T_{\text{ext}})$.

For a given T_{ext}, we can calculate the establishment probability of a single double mutant based on a time-inhomogeneous branching process with death rate $\frac{1}{2} - \frac{\hat{s}_{\text{eff}}(t)}{2}$ and birth rate $\frac{1}{2} + \frac{\hat{s}_{\text{eff}}(t)}{2}$ with $\hat{s}_{\text{eff}}(t)$ defined by

$$\hat{s}_{\text{eff}}(t) = \begin{cases} \ln((1+s_{AB})(1-r)) & t \leq T_{\text{ext}}, \\ \ln(1+s_{AB}) & t > T_{\text{ext}} \end{cases} \tag{S1.26}$$

(see Eq. (S1.10)). This gives for $t < T_{\text{ext}}$:

$$p_{\text{est}}^{(AB)}(t|T_{\text{ext}}) = \frac{2}{1 + I(t, T_{\text{ext}})} \tag{S1.27}$$

with

$$\begin{aligned} I(t, T_{\text{ext}}) &= \int_t^\infty e^{-\int_t^T \hat{s}_{\text{eff}}(\tau)\mathrm{d}\tau} \mathrm{d}T \\ &= \frac{1}{s_1} - \left(\frac{1}{s_1} - \frac{1}{s_2} \right) e^{-s_1(T_{\text{ext}} - t)}, \end{aligned} \tag{S1.28}$$

where s_1 and s_2 are given by $\hat{s}_{\text{eff}}$ before and after extinction of the wildtype respectively. For $t \geq T_{\text{ext}}$, the establishment probability is given by Eq. (S1.20).

Over all possible extinction times, we get

$$p_{\text{est}}^{(AB)}(t) = \int_t^\infty p(T_{\text{ext}}) \frac{2}{1 + I(t, T_{\text{ext}})} \mathrm{d}T_{\text{ext}} + \int_0^t p(T_{\text{ext}}) \frac{2\ln(1+s_{AB})}{1 + \ln(1+s_{AB})} \mathrm{d}T_{\text{ext}}. \tag{S1.29}$$

The numerical evaluation of integrals is done in Mathematica (Wolfram Research, Champaign, USA).

File S2: No recombination

For complete linkage, approximations have been derived in Iwasa *et al.* (2003, 2004). These approximations model all allele frequencies in the standing genetic variation deterministically. We extend these results by a stochastic treatment of the number of double mutants in the standing genetic variation.

The distribution of genotypes in the standing genetic variation. In principle, the number of single and double mutants in the population can be modeled as a two-type branching process with immigration. However, analytical solutions for the p.g.f. are not easily derived. We therefore propose two simpler approximations to estimate the contribution of the standing genetic variation for rescue. (1) If the population size is small, double mutants in the standing genetic variation can often be neglected; the number of single mutants is subject to stochasticity. The probability generating functions F_{Ab} and F_{aB} are given by Eq. (S1.13). (2) If the population size is large, the number of single mutant types is well approximated by their expected value (Eq. (S1.14)). The probability generating function for the number of double mutants F_{AB} is then given by Eq. (S1.17).

Establishment probability of the rescue mutant. After the change in the environment, a lineage initiated by one individual of type AB survives with probability $p_{\text{est}}^{(AB)}$ as given by Eq. (S1.20). A lineage that is founded by a single individual of type Ab (or aB) survives with probability $p_{\text{est}}^{(Ab)}$ as given by Eq. (S1.21). These results do not depend on the dynamics of the wildtype when $r = 0$ because of our assumption of a hard carrying capacity (no density dependence until $N \geq N_0$).

The probability of evolutionary rescue. We first consider the case that the number of double mutants before the change in the environment can be ignored. Rescue can now either pass via single mutants from the standing genetic variation or via newly generated single mutants. The number of successful offspring of a single type Ab individual is Poisson distributed with parameter $(1 + s_{Ab})p_{\text{est}}^{(Ab)}$. If n_{Ab} individuals of type Ab are present at the time of environmental change, they hence do not establish a permanent lineage with probability $\exp\left[-n_{Ab}(1 + s_{Ab})p_{\text{est}}^{(Ab)}\right]$. It remains to average over the distribution of n_{Ab}, for which one can conveniently use the p.g.f. F_{Ab}, Eq. (S1.13) (analogous for type aB). In order to determine the number of single mutants that get generated after the environmental change, we assume that the decay of the wildtype population size can be well described deterministically by $n_{ab}(t) \approx N_0(1 + s_{ab})^t$ (cf. Orr and Unckless, 2008; Uecker *et al.*, 2014). The number of de-novo generated single mutants is then given by $\sum_{t=0}^{\infty} u n_{ab}(t)(1 + s_{Ab}) \approx \frac{uN_0}{-s_{ab}}(1 + s_{Ab})$. With this, we obtain:

$$P_{\text{rescue}} = 1 - F_{Ab}(e^{-(1+s_{Ab})p_{\text{est}}^{(Ab)}})F_{aB}(e^{-(1+s_{aB})p_{\text{est}}^{(aB)}})e^{-\frac{uN_0}{-s_{ab}}(1+s_{Ab})p_{\text{est}}^{(Ab)} - \frac{uN_0}{-s_{ab}}(1+s_{aB})p_{\text{est}}^{(aB)}}. \qquad \text{(S2.1)}$$

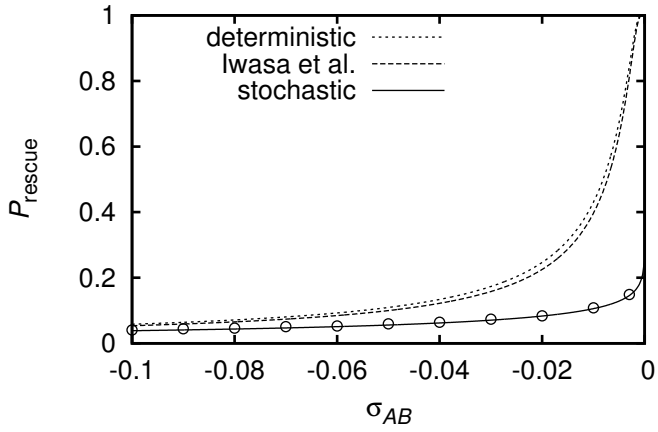

Fig. S2.1: **Probability of evolutionary rescue as a function of σ_{AB}.** The theoretical predictions are based on Eq. (S2.2) (solid line), Iwasa *et al.* (2003, 2004) (long-dashed line), and Eq. (S2.3) (short-dashed line). Parameter values: $\sigma_{Ab} = \sigma_{aB} = -0.01$, $s_{Ab} = s_{aB} = s_{ab} = -0.5$, $s_{AB} = 0.15$, $u = 10^{-5}$, $N_0 = 10^6$. Symbols denote simulation results. Each simulation point is the average of 10^5 replicates.

If single mutants are frequent and we describe double mutants stochastically, using the expected values $\bar{n}_{Ab}$ and $\bar{n}_{aB}$, we have:

$$P_{\text{rescue}} = 1 - F_{AB}(e^{-(1+s_{AB})p_{\text{est}}^{(AB)}}) e^{-u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})p_{\text{est}}^{(AB)}} e^{-\bar{n}_{Ab}(1+s_{Ab})p_{\text{est}}^{(Ab)} - \bar{n}_{aB}(1+s_{aB})p_{\text{est}}^{(aB)}} \times e^{-\frac{uN_0}{-s_{ab}}(1+s_{Ab})p_{\text{est}}^{(Ab)} - \frac{uN_0}{-s_{ab}}(1+s_{aB})p_{\text{est}}^{(aB)}}. \tag{S2.2}$$

If we can treat the number of double mutants deterministically, we obtain:

$$P_{\text{rescue}} = 1 - e^{-(1+s_{AB})\bar{n}_{AB}p_{\text{est}}^{(AB)}} e^{-u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})p_{\text{est}}^{(AB)}} e^{-\bar{n}_{Ab}(1+s_{Ab})p_{\text{est}}^{(Ab)} - \bar{n}_{aB}(1+s_{aB})p_{\text{est}}^{(aB)}} \times e^{-\frac{uN_0}{-s_{ab}}(1+s_{Ab})p_{\text{est}}^{(Ab)} - \frac{uN_0}{-s_{ab}}(1+s_{aB})p_{\text{est}}^{(aB)}} \tag{S2.3}$$

with

$$\bar{n}_{AB} = \frac{u(\bar{n}_{Ab} + \bar{n}_{aB})}{-\sigma_{AB}}(1 + \sigma_{AB}). \tag{S2.4}$$

Comparison to Iwasa et al. (2003, 2004). We can compare our approximations to the approximation derived in Iwasa *et al.* (2003, p. 2574) and Iwasa *et al.* (2004, Eq. 9), who describe all allele frequencies prior to the environmental change deterministically (derived as the stationary solution of a system of differential equations). Consequently, as can be seen from Fig. S2.1, the approximation is in good agreement with Eq. (S2.3) (up to minor deviations due to details in the model and the analysis). Both strongly overestimate the real rescue probability in Fig. S2.1. The reason is that the number of double mutants in the standing genetic variation

– from which rescue mainly occurs in the parameter regime shown in the figure – is subject to strong fluctuations. This matters mainly for weakly deleterious double mutants: Then, the average number of double mutants is high enough to provide a population with a decent chance to survive, and the deterministic approximation assumes that each replicate population contains this average number of double mutants. Stochastically, however, some replicate populations have a very high chance to survive (but a single population can only get rescued once; the very high number of double mutants is hence redundant), while most of them contain no double mutants at all and go extinct.

File S3: The role of recombination

From now on, we assume that the population is large enough that we can approximate the number of Ab and aB mutants in the standing genetic variation by their expected number, Eq. (S1.14). For the number of double mutants prior to the environmental change, we use F_{AB}, Eq. (S1.17). In order to keep the equations simple, we usually assume $\sigma_{Ab} = \sigma_{aB} = \sigma$. Generalization to unequal selection coefficients for single mutants before the environmental change is straightforward.

S3.1 Single mutants are lethal in the new environment

The wildtype is lethal too. In the absence of any other types, a single rescue type individual establishes a permanent lineage with probability $p_{\text{est}}^{(AB)}$, Eq. (S1.20). In the first generation after the switch, with our choice of the life cycle (mutation and recombination before selection), the wildtype and the single mutants are, however, still present in the population (leading to the generation and deletion of AB mutants). A single rescue type individual present at the time of environmental change will hence not establish a permanent lineage with probability $\exp\left[-p_{\text{est}}^{(AB)}(1+s_{AB})(1-r)\right]$, and the probability that no new successful lineage is generated by recombination or mutation in this first generation is given by $\exp\left[-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N}(1+s_{AB}) + u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}\right]$. With this, the probability of evolutionary rescue is given by

$$P_{\text{rescue}} = 1 - F_{AB}(e^{-(1+s_{AB})(1-r)p_{\text{est}}^{(AB)}}) \times e^{-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}(1+s_{AB})+u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}}. \qquad \text{(S3.1)}$$

With $p_{\text{est}}^{(AB)} \approx 2s_{AB}$ and $\sigma_{Ab} = \sigma_{aB} = \sigma$, we can approximate

$$\begin{aligned}
&F(e^{-(1+s_{AB})(1-r)p_{\text{est}}^{(AB)}}) \approx F(1-2s_{AB}(1-r)) \\
&= \left(1 + \frac{2s_{AB}(1+\sigma_{AB})(1-r)^2}{2(1-r)(1+\sigma_{AB}) - 2s_{AB}(1-r)^2(1+\sigma_{AB}) - 2}\right)^{\frac{2(1+\sigma_{AB})\left[r\frac{u^2N_0}{\sigma^2}(1+\sigma)^2 + \frac{u^2N_0}{-\sigma}(1+\sigma)(1-r)\right]}{(1+\sigma_{AB})(1-r)}} \\
&= \left(1 + \frac{s_{AB}(1-r)^2}{-s_{AB}(1-r)^2 + (1-r)\sigma_{AB} - 1}\right)^{-\frac{2u^2N_0}{\sigma^2}\left[2\sigma - \frac{r}{1-r}\right]} \\
&\approx \left(\frac{r - (2\sigma + E_1)(1-r)}{s_{AB}(1-r)^2 + r - (2\sigma+E_1)(1-r)}\right)^{-\frac{2u^2N_0}{\sigma^2}\left[2\sigma - \frac{r}{1-r}\right]} \\
&\approx \left(\frac{r - 2\sigma - E_1}{s_{AB}(1-r)^2 + r - 2\sigma - E_1}\right)^{-\frac{2u^2N_0}{\sigma^2}\left[2\sigma - \frac{r}{1-r}\right]},
\end{aligned} \qquad \text{(S3.2)}$$

where the first approximation is a series expansion of the exponential function up to first order in the exponent and the second approximation is based on dropping higher order terms in σ_{AB} and σ in the enumerator, the denominator, and the exponent. The approximation in the last line consists in approximating $r-(1-r)(2\sigma+E_1) \approx r-2\sigma-E_1$ since the second term only matters when r is small, i.e. when $1-r \approx 1$. If we furthermore ignore new mutations after the switch in the environment, we obtain:

$$P_{\text{rescue}} \approx 1 - \left(\frac{r-2\sigma-E_1}{s_{AB}(1-r)^2+r-2\sigma-E_1}\right)^{-\frac{2u^2N_0}{\sigma^2}\left[2\sigma-\frac{r}{1-r}\right]} e^{-2s_{AB}r\frac{u^2N_0}{\sigma^2}}. \tag{S3.3}$$

If we do not take stochasticity in the number of double mutants in the standing genetic variation into account, we get

$$\begin{aligned} P_{\text{rescue}}^{\text{det}} &= 1 - e^{-\langle n_{AB}\rangle(1+s_{AB})(1-r)p_{\text{est}}^{(AB)}} \times e^{-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N}(1+s_{AB})+u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}} \\ &\approx 1 - e^{-2\frac{u^2N_0}{\sigma^2}s_{AB}\left[1-\frac{(1-r)(2-2\sigma-E_1)}{r-2\sigma-E_1}\right]} \\ &\approx 1 - e^{-2s_{AB}\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1}}, \end{aligned} \tag{S3.4}$$

where the first approximation makes use of the approximation for $\langle n_{AB}\rangle$ (Eq. S1.19) and furthermore uses $p_{\text{est}}^{(AB)} \approx 2s_{AB}$ and $1+s_{AB} \approx 1$ and ignores new mutations from generation 0 to 1.

With this, we can compare the probability of evolutionary rescue (1) without epistasis and without drift (Eq. S3.4 with $E_1 = 0$), (2) without epistasis but with drift (Eq. S3.1 with $E_1 = 0$), (3) with epistasis but without drift (Eq. S3.4 with $E_1 \neq 0$), and (4) with epistasis and with drift (Eq. S3.1 with $E_1 \neq 0$). Fig. S3.1 shows all four cases. Note that the establishment of the rescue type after the environmental change is in any case subject to strong stochasticity.

Last, we want to estimate the influence of drift on the rescue probability

$$d = \frac{P_{\text{rescue}} - P_{\text{rescue}}^{\text{det}}}{P_{\text{rescue}}^{\text{det}}}. \tag{S3.5}$$

For this, we approximate by a Taylor expansion up to leading order in s_{AB} (and similar approximations as in Eq. S3.4):

$$\begin{aligned} P_{\text{rescue}} - P_{\text{rescue}}^{\text{det}} &\approx \left(e^{-2s_{AB}\langle n_{AB}\rangle(1-r)} - \langle e^{-2s_{AB}n_{AB}(1-r)}\rangle\right) e^{-2s_{AB}r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}} \\ &\approx -2s_{AB}^2(1-r)^2\text{Var}[n_{AB}] + \mathcal{O}\left(s_{AB}^3\right). \end{aligned} \tag{S3.6}$$

This leaves us with

$$\begin{aligned} d &\approx -\frac{s_{AB}(1-r)^2 \mathrm{Var}[n_{AB}]}{(1-r)\langle n_{AB}\rangle + r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}} + \mathcal{O}\left(s_{AB}^2\right) \\ &= -\frac{-s_{AB}(1-r)^2\frac{\mathrm{Var}[n_{AB}]}{\langle n_{AB}\rangle}}{(1-r) + r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0\langle n_{AB}\rangle}} + \mathcal{O}\left(s_{AB}^2\right) \\ &\approx \frac{\mathrm{Var}[n_{AB}]}{\langle n_{AB}\rangle} \cdot \frac{-s_{AB}(1-r)^2}{1 + r\frac{E_1}{r-2\sigma}} + \mathcal{O}\left(s_{AB}^2\right). \end{aligned} \tag{S3.7}$$

For the last line, we used Eq. (S1.19) and $\bar{n}_{Ab} = \bar{n}_{aB} \approx -\frac{uN_0}{\sigma}$. For the ratio of variance to mean, we obtain:

$$\begin{aligned} \frac{\mathrm{Var}[n_{AB}]}{\langle n_{AB}\rangle} &= \frac{F''_{AB}(1) + F'_{AB}(1) - F'_{AB}(1)^2}{F'_{AB}(1)} \\ &= \frac{1}{2}\left(1 + \frac{1}{r(1+\sigma_{AB}) - \sigma_{AB}}\right), \end{aligned} \tag{S3.8}$$

which is a decreasing function of r, i.e., the relative importance of drift decreases with r. Note that the variance itself depends on epistasis and is not decreasing over the entire parameter range (it can be increasing, decreasing, or be non-monotonic).

For $|\sigma|$ and $|\sigma_{AB}|$ small, we can further approximate

$$d \approx -\frac{\mathrm{Var}[n_{AB}]}{\langle n_{AB}\rangle} s_{AB}(1-r)^2 \approx -\frac{1}{2}(1-r)^2(1+r)\frac{s_{AB}}{r-\sigma_{AB}}. \tag{S3.9}$$

Although the approximation deviates from the exact result for small r, we can read off the qualitative behavior: d is negative and monotonically increasing with r, i.e., the larger r, the less drift reduces P_{rescue}. We can distinguish two regimes: (1) If $|\sigma_{AB}| \gg s_{AB}$, drift does not play a significant role, irrespective of r. (2) If $|\sigma_{AB}| \ll s_{AB}$, drift has a significant influence unless $r \gg s_{AB}$.

The wildtype remains. If the wildtype population size decays slowly after the environmental change, the establishment probability of a single rescue mutant is well approximated by Eq. (S1.23). Analogous to before, we again have

$$P_{\mathrm{rescue}} = 1 - F_{AB}(e^{-(1+s_{AB})(1-r)p_{\mathrm{est}}^{(AB)}}) \times e^{-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}(1+s_{AB}) + u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})(1-r)\right)p_{\mathrm{est}}^{(AB)}}. \tag{S3.10}$$

Actually, $e^{-(1+s_{AB})(1-r)(1-q_{AB})} = q_{AB}$ (where q_{AB} is the exact extinction probability of a branching process with Poisson distributed offspring numbers with mean $(1+s_{AB})(1-r)$), and so we could simply use $F_{AB}(1-p_{\mathrm{est}}^{(AB)})$. Since we use an approximation for q_{AB} (which is our approximation $1-p_{\mathrm{est}}^{(AB)}$), we prefer the above form for consistency with the previous paragraph.

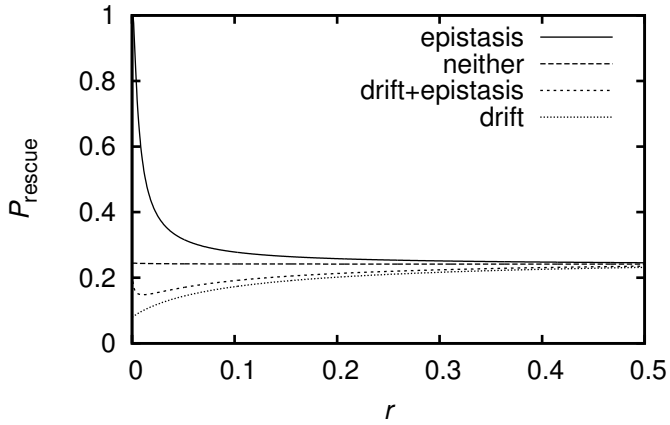

Fig. S3.1: **Probability of evolutionary rescue as a function of recombination (cf. Fig. 1).** The curves are based on Eq. (S3.1) (drift) and Eq. (S3.4) (no drift). Paramater values are: $\sigma_{AB} = -0.0199$ (no epistasis) and $\sigma_{AB} = -0.0001$ (epistasis), $\sigma_{Ab} = \sigma_{aB} = -0.01$, $u = 10^{-5}$, $N_0 = 10^6$, $s_{AB} = 0.15$, $s_{Ab} = s_{aB} = s_{ab} = -1$.

As before, we can derive an approximation, ignoring stochasticity in the number of double mutants

$$P_{\text{rescue}}^{\text{det}} = 1 - e^{-\langle n_{AB}\rangle(1+s_{AB})(1-r)p_{\text{est}}^{(AB)}} \times e^{-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N}(1+s_{AB})+u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}}$$
$$\approx \begin{cases} 1 - e^{-2\frac{u^2N_0}{\sigma^2}(s_{AB}-r)\left[1-\frac{(2-2\sigma-E_1)(1-r)}{r-2\sigma-E_1}\right]} \approx 1 - e^{-2(s_{AB}-r)\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1}} & \text{if} \quad s_{AB} - r > 0, \\ 0 & \text{else,} \end{cases} \tag{S3.11}$$

where we approximate $p_{\text{est}}^{(AB)} \approx \max[2(s_{AB} - r), 0]$.

The wildtype is quite unfit. If the wildtype is not very fit, we need to resort to the more accurate approximation Eq. (S1.29) for the establishment probability of the double mutant. For the probability of rescue, we obtain as before:

$$P_{\text{rescue}} = 1 - F_{AB}(e^{-(1+s_{AB})(1-r)p_{\text{est}}^{(AB)}(1)}) \times e^{-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}(1+s_{AB})+u(n_{Ab}+n_{aB})(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}(1)}. \tag{S3.12}$$

Sensitivity of the approximation. How sensitive are the approximations to the assumption of lethality of the single mutants? Fig. S3.2 compares the approximations (assuming $s_{Ab} = s_{aB} = -1$) to simulations with $s_{Ab} = s_{aB} = -0.99$ (Panel A) and $s_{Ab} = s_{aB} = -0.9$ (Panel B). The fitter the wildtype the less sensitive is the approximation to deviations from strict lethality of the single mutants. For a lethal wildtype, even a slight increase in the fitness of mutants above lethality drastically increases P_{rescue}.

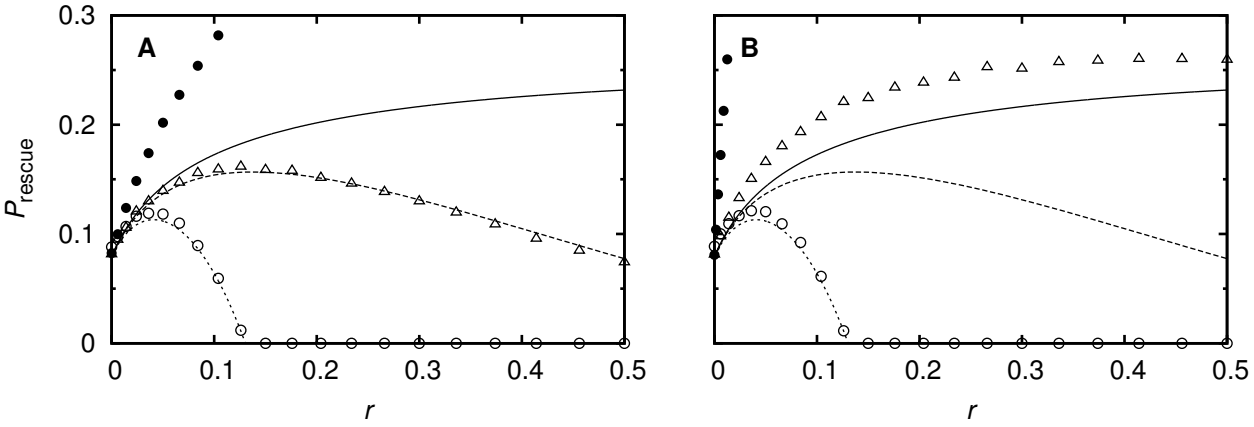

Fig. S3.2: **Probability of evolutionary rescue as a function of recombination.** The figure is identical to Fig. 1 except for that we set $s_{Ab} = s_{aB} = -0.99$ (Panel A) and $s_{Ab} = s_{aB} = -0.9$ (Panel B) in the simulations. The growth parameter of the wildtype is $s_{ab} = -1$ (solid lines, filled circle), $s_{ab} = -0.99$ (dashed line, triangles), $s_{ab} = -0.005$ (dotted line, empty circles). Circles and triangles denote simulation results. Each simulation point is the average of 10^5 replicates.

S3.2 One single mutant is viable, the other lethal

Let us now consider the situation $s_{Ab} > -1$ and $s_{aB} = -1$ after the environmental change.

The wildtype is lethal. The presence of one of the single mutant types after the environmental change opens up a new rescue pathway: new double mutants can be generated by mutation after generation 0. Analogous to before, the probability that the population is rescued via this pathway is given by

$$1 - e^{-(\bar{n}_{Ab}+uN_0)(1+s_{Ab})p_{\text{est}}^{(Ab)}} \tag{S3.13}$$

with $p_{\text{est}}^{(Ab)}$ given by Eq. (S1.21). Combination with Eq. (S3.1) yields the total probability of evolutionary rescue:

$$\begin{aligned} P_{\text{rescue}} = 1 - F_{AB}(e^{-(1+s_{AB})(1-r)p_{\text{est}}^{(AB)}}) \times e^{-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}(1+s_{AB})+u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}} \\ \times e^{-(\bar{n}_{Ab}+uN_0)(1+s_{Ab})p_{\text{est}}^{(Ab)}}. \end{aligned} \tag{S3.14}$$

We can estimate the respective significance of the contributions by a comparison of Eq. (S3.13) with Eq. (S3.4), assuming $\sigma_{Ab} = \sigma_{aB}$. Approximating $\bar{n}_{Ab} \approx \frac{uN_0}{-\sigma_{Ab}}$ and $1 + s_{Ab} \approx 1$ and ignoring the term that accounts for new mutations ($\sim uN_0$) in Eq. (S3.13) and setting $E_1 = 0$ in Eq. (S3.4), we arrive at the condition

$$p_{\text{est}}^{(Ab)} > 2\frac{us_{AB}}{-\sigma_{Ab}} \tag{S3.15}$$

for the contribution of new rescue mutations after the environmental change being larger than the contribution by double mutants from the standing genetic variation. With the last approximation for $p_{\text{est}}^{(Ab)}$ in Eq. (S1.21), this condition simplifies to

$$\frac{2us_{Ab}}{-s_{Ab}} > \frac{2us_{Ab}}{-\sigma_{Ab}} \quad \Leftrightarrow -\sigma_{Ab} > -s_{Ab}. \tag{S3.16}$$

If $s_{Ab} > 0$, rescue is not contingent on the generation of the double mutant. Depending on the mutation rate and the fitness effects of mutations, generation of the double mutant might still help rescue or be negligible. In the latter case, results from single step rescue apply (Orr and Unckless, 2008; Bell and Collins, 2008; Uecker *et al.*, 2014). Formation of the double mutant after the environmental change cannot be ignored in Eq. (S3.13) if

$$\begin{aligned}
& 2s_{Ab} \ll p_{\text{est}}^{(Ab)} \\
\Leftrightarrow \quad & 2s_{Ab} \ll s_{Ab} - u + \sqrt{(s_{Ab}-u)^2 + 4s_{AB}u} \\
\Leftrightarrow \quad & s_{Ab} + u \ll \sqrt{(s_{Ab}-u)^2 + 4s_{AB}u} \\
\overset{s_{Ab} \gg u}{\Leftrightarrow} \quad & s_{Ab} \ll \sqrt{s_{Ab}^2 + 4s_{AB}u} = s_{Ab} \cdot \sqrt{1 + \frac{4s_{AB}u}{s_{Ab}^2}} \\
\Leftrightarrow \quad & 4s_{AB}u \gg s_{Ab}^2.
\end{aligned} \tag{S3.17}$$

Altogether, generation of the double mutant cannot be ignored if

$$\begin{aligned}
& 2s_{Ab}\frac{uN_0}{-\sigma} \ll p_{\text{est}}^{(Ab)}\frac{uN_0}{-\sigma} + 2s_{AB}\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \\
\Leftrightarrow \quad & 2s_{Ab} \ll s_{Ab} - u + \sqrt{(s_{Ab}-u)^2 + 4s_{AB}u} + 2s_{AB}\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \\
\Leftrightarrow \quad & s_{Ab} + u \ll \sqrt{(s_{Ab}-u)^2 + 4s_{AB}u} + 2s_{AB}\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \\
\overset{s_{Ab} \gg u}{\Leftrightarrow} \quad & s_{Ab} \ll s_{Ab} \cdot \sqrt{1 + \frac{4s_{AB}u}{s_{Ab}^2}} + 2s_{AB}\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \\
\Leftrightarrow \quad & 4s_{AB}u \gg s_{Ab}^2 \quad \text{or} \quad 2s_{AB}\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \gg s_{Ab}.
\end{aligned} \tag{S3.18}$$

The wildtype is at least as fit as the viable single mutant. Viability of the wildtype has two consequences: (1) The double mutant can be broken up by recombination. (2) The wildtype can generate new *Ab* mutants on its course to extinction. Modeling the wildtype deterministically, we obtain for the probability of rescue by de-novo generated double mutants

$$1 - e^{-\bar{n}_{Ab}(1+s_{Ab})p_{\text{est}}^{(Ab)}} \times e^{-\frac{uN_0}{-s_{ab}}(1+s_{Ab})p_{\text{est}}^{(Ab)}}. \tag{S3.19}$$

Combination with Eq. (S3.10) yields again the total probability of evolutionary rescue:

$$P_{\text{rescue}} = 1 - F_{AB}(e^{-(1+s_{AB})(1-r)p_{\text{est}}^{(AB)}}) \times e^{-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}(1+s_{AB})+u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}} \times e^{-(\bar{n}_{Ab}+\frac{uN_0}{-s_{ab}})(1+s_{Ab})p_{\text{est}}^{(Ab)}}. \tag{S3.20}$$

As before, we can compare the different pathways to rescue, (a) from double mutants from the standing genetic variation, (b) mutation of single mutants from the standing genetic variation after the change in the environment, (c) complete de-novo generation via the wildtype after the environmental switch. Pathway (c) is more important than pathway (b) if

$$-s_{ab} < -\sigma_{Ab}. \tag{S3.21}$$

Pathway (b) is more important than pathway (a) if

$$-s_{Ab} < -\sigma_{Ab}. \tag{S3.22}$$

If $s_{Ab} > 0$, analogous to the previous paragraph, formation of the double mutant after the environmental change cannot be ignored if

$$\begin{aligned}
& 2s_{Ab} \ll p_{\text{est}}^{(Ab)} \\
\Leftrightarrow\quad & 2s_{Ab} \ll s_{Ab} - u + \sqrt{(s_{Ab}-u)^2 + 4\max[(s_{AB}-r),0]u} \\
\Leftrightarrow\quad & s_{Ab} + u \ll \sqrt{(s_{Ab}-u)^2 + 4\max[(s_{AB}-r),0]u} \\
\overset{s_{Ab}\gg u}{\Leftrightarrow}\quad & s_{Ab} \ll \sqrt{s_{Ab}^2 + 4s_{AB}u} = s_{Ab}\cdot\sqrt{1+\frac{4\max[(s_{AB}-r),0]u}{s_{Ab}^2}} \\
\Leftrightarrow\quad & 4\max[(s_{AB}-r),0]u \gg s_{Ab}^2.
\end{aligned} \tag{S3.23}$$

Altogether, it cannot be ignored if

$$\begin{aligned}
& 2s_{Ab}\frac{uN_0}{-\sigma} \ll p_{\text{est}}^{(Ab)}\frac{uN_0}{-\sigma} + \max[2(s_{AB}-r),0]\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \\
\Leftrightarrow\quad & 2s_{Ab} \ll s_{Ab} - u + \sqrt{(s_{Ab}-u)^2 + 4\max[(s_{AB}-r),0]u} + \max[2(s_{AB}-r),0]\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \\
\Leftrightarrow\quad & s_{Ab} + u \ll \sqrt{(s_{Ab}-u)^2 + 4\max[(s_{AB}-r),0]u} + \max[2(s_{AB}-r),0]\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \\
\overset{s_{Ab}\gg u}{\Leftrightarrow}\quad & s_{Ab} \ll s_{Ab}\cdot\sqrt{1+\frac{4\max[(s_{AB}-r),0]u}{s_{Ab}^2}} + \max[2(s_{AB}-r),0]\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \\
\Leftrightarrow\quad & 4\max[(s_{AB}-r),0]u \gg s_{Ab}^2 \quad \text{or} \quad \max[2(s_{AB}-r),0]\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} \gg s_{Ab}.
\end{aligned} \tag{S3.24}$$

S3.3 Both single mutants are viable

Finally, we consider the case $s_{Ab} = s_{aB} = s > -1$. With $\sigma_{Ab} = \sigma_{aB} = \sigma$, deterministically, the number of Ab mutants and aB mutants is hence equal at any point of time. In the following, we formulate equations in terms of type Ab.

The wildtype is lethal. Ignoring recombination, from generation 0 to generation 1, the number of Ab individuals changes to

$$n_{Ab}(1) = (\bar{n}_{Ab}(1-2u) + uN_0)\,(1+s). \tag{S3.25}$$

From then on, it evolves according to the recursive equation

$$\begin{aligned} n_{Ab}(t+1) &= (1+s)(1-2u)\left(n_{Ab}(t) - r\frac{n_{Ab}(t)n_{aB}(t)}{n_{Ab}(t)+n_{aB}(t)}\right) \\ &= (1+s)(1-2u)\left(n_{Ab}(t) - \frac{r}{2}n_{Ab}(t)\right), \end{aligned} \tag{S3.26}$$

where the second line holds since $n_{Ab}(t) = n_{aB}(t)$. With this, we have

$$n_{Ab}(t+1) = n_{Ab}(1)\left((1+s)(1-2u)\left(1-\frac{r}{2}\right)\right)^t. \tag{S3.27}$$

From generation 1 on, the number of newly generated AB individuals follows a Poisson distribution with parameter

$$\left(u(n_{Ab}(t)+n_{aB}(t)) + \frac{r}{2}n_{Ab}(t)\right)(1+s_{AB}). \tag{S3.28}$$

Putting all together and using again $n_{Ab}(t) = n_{aB}(t)$, we obtain for rescue from generation 1 on:

$$1 - e^{-\sum_{t=0}^{\infty}\left(2u+\frac{r}{2}\right)n_{Ab}(t+1)(1+s_{AB})p_{\text{est}}^{(AB)}}. \tag{S3.29}$$

With

$$\sum_{t=0}^{\infty} n_{Ab}(t+1) = \sum_{t=0}^{\infty} n_{Ab}(1)\left((1+s)(1-2u)\left(1-\frac{r}{2}\right)\right)^t = n_{Ab}(1)\frac{1}{1-(1+s)(1-2u)\left(1-\frac{r}{2}\right)}, \tag{S3.30}$$

this yields

$$1 - e^{-\left(\frac{(1+s_{AB})\left(2u+\frac{r}{2}\right)n_{Ab}(1)}{1-(1+s)(1-2u)\left(1-\frac{r}{2}\right)}\right)p_{\text{est}}^{(AB)}} \approx 1 - e^{-2s_{AB}\frac{\frac{r}{2}\frac{uN_0(1+s)}{-\sigma}}{\frac{r}{2}+2u-s}}. \tag{S3.31}$$

Combining with Eq. (S3.1), we obtain for the total probability of evolutionary rescue

$$\begin{aligned} P_{\text{rescue}} = 1 - F_{AB}(e^{-(1+s_{AB})(1-r)p_{\text{est}}^{(AB)}}) &\times e^{-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}(1+s_{AB})+u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}} \\ &\times e^{-\left(\frac{(1+s_{AB})\left(2u+\frac{r}{2}\right)n_{Ab}(1)}{1-(1+s)(1-2u)\left(1-\frac{r}{2}\right)}\right)p_{\text{est}}^{(AB)}}. \end{aligned} \tag{S3.32}$$

The wildtype is as fit as the single mutants. As a second scenario, we consider the special case $s_{ab} = s_{Ab} = s_{aB} = s$. If we ignore mating between single mutants (note that unlike in the previous scenario, they are now relatively rare), we obtain for the deterministic dynamics

$$n_{ab}(t+1) = (1+s)(n_{ab}(t) - 2un_{ab}(t)), \tag{S3.33a}$$

$$n_{Ab}(t+1) = (1+s)(n_{Ab}(t) + un_{ab}(t)), \tag{S3.33b}$$

$$n_{aB}(t+1) = (1+s)(n_{aB}(t) + un_{ab}(t)) \tag{S3.33c}$$

with the solutions

$$n_{ab}(t) = \bar{n}_{ab}((1+s)(1-2u))^t, \tag{S3.34a}$$

$$n_{Ab}(t) = n_{aB}(t) = \frac{1}{2}\left(N_0(1+s)^t - \bar{n}_{ab}((1+s)(1-2u))^t\right), \tag{S3.34b}$$

and $\bar{n}_{ab} = N_0 - \bar{n}_{Ab} - \bar{n}_{aB}$. Type AB is generated at rate

$$r\frac{n_{Ab}(t)n_{aB}(t)}{N(t)}(1+s_{AB}) + u(n_{Ab}(t) + n_{aB}(t))(1+s_{AB})\,(1-r) \tag{S3.35}$$

and establishes with probability $p_{\text{est}}^{(AB)}$ as given by Eq. (S1.23). This yields for the probability of evolutionary rescue via this pathway

$$1 - e^{-\sum_{t=1}^{\infty}\left(r\frac{n_{Ab}(t)n_{aB}(t)}{N(t)}(1+s_{AB}) + u(n_{Ab}(t)+n_{aB}(t))(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}}. \tag{S3.36}$$

Evaluating the sums yields

$$\begin{aligned}\sum_{t=1}^{\infty}&\frac{n_{Ab}(t)n_{aB}(t)}{N(t)}\\ &= -\frac{N_0}{4s} - \frac{N_0 - \bar{n}_{Ab} - \bar{n}_{aB}}{2(1-(1+s)(1-2u))} + \frac{(N_0 - \bar{n}_{Ab} - \bar{n}_{aB})^2}{4N_0}\frac{1}{1-(1+s)(1-2u)^2} - \frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0},\end{aligned} \tag{S3.37a}$$

$$\sum_{t=1}^{\infty}(n_{Ab}(t) + n_{aB}(t)) = -\frac{N_0}{s} - \frac{N_0 - \bar{n}_{Ab} - \bar{n}_{aB}}{1-(1+s)(1-2u)} - \bar{n}_{Ab} - \bar{n}_{aB}. \tag{S3.37b}$$

Putting it all together, we obtain:

$$\begin{aligned}P_{\text{rescue}} = 1 - F(e^{-(1+s_{AB})(1-r)p_{\text{est}}^{(AB)}}) &\times e^{-\left(r\frac{\bar{n}_{Ab}\bar{n}_{aB}}{N_0}(1+s_{AB}) + u(\bar{n}_{Ab}+\bar{n}_{aB})(1+s_{AB})(1-r)\right)p_{\text{est}}^{(AB)}}\\ &\times e^{-\left(r(1+s_{AB})\sum_{t=1}^{\infty}\frac{n_{Ab}(t)n_{aB}(t)}{N(t)} + u(1-r)(1+s_{AB})\sum_{t=1}^{\infty}(n_{Ab}(t)+n_{aB}(t))\right)p_{\text{est}}^{(AB)}}.\end{aligned} \tag{S3.38}$$

The wildtype is fitter than the single mutants. If $s_{Ab} = s_{aB} = s$ and $s_{ab} > s$, we can proceed as in the previous section. The dynamics of the wildtype population are again given by

$$n_{ab}(t) = \bar{n}_{ab}(1 + s_{ab})^t(1 - 2u)^t. \tag{S3.39}$$

The dynamics of the single mutants follow

$$n_{Ab}(t + 1) = n_{aB}(t + 1) = (1 + s)(n_{Ab}(t) + un_{ab}(t)), \tag{S3.40}$$

yielding

$$n_{Ab}(t) = \frac{(uN_0(1 + s) + \bar{n}_{Ab}(s - s_{ab})(1 - 2u))(1 + s)^t - u\bar{n}_{ab}(1 + s)(1 + s_{ab})^t(1 - 2u)^t}{s - s_{ab} + 2u(1 + s_{ab})}. \tag{S3.41}$$

With the abbreviations

$$\begin{aligned} C &:= s - s_{ab} + 2u(1 + s_{ab}), \\ \alpha &:= u\bar{n}_{ab}(1 + s), \\ \beta &:= uN_0(1 + s) + \bar{n}_{Ab}(s - s_{ab})(1 - 2u), \end{aligned} \tag{S3.42}$$

and $N(t) \approx n_{ab}(t)$ we obtain

$$\sum_{t=1}^{\infty}(n_{Ab}(t) + n_{aB}(t)) = \frac{\beta\sum_{t=1}^{\infty}(1 + s)^t - \alpha\sum_{t=1}^{\infty}(1 + s_{ab})^t(1 - 2u)^t}{C} = \frac{\beta\frac{1+s}{-s} - \alpha\frac{(1+s_{ab})(1-2u)}{1-(1+s_{ab})(1-2u)}}{C}, \tag{S3.43a}$$

$$\begin{aligned} \sum_{t=1}^{\infty}\frac{n_{Ab}(t)n_{aB}(t)}{N(t)} &= \frac{\alpha^2(1 + s_{ab})^{2t}(1 - 2u)^{2t} - 2\alpha\beta(1 + s_{ab})^t(1 - 2u)^t(1 + s)^t + \beta^2(1 + s)^{2t}}{C^2\bar{n}_{ab}(1 + s_{ab})^t(1 - 2u)^t} \\ &= \frac{1}{C^2\bar{n}_{ab}}\left(\alpha^2\frac{(1 + s_{ab})(1 - 2u)}{1 - (1 + s_{ab})(1 - 2u)} - 2\alpha\beta\frac{1 + s}{-s} + \beta^2\frac{(1 + s)^2}{(1 + s_{ab})(1 - 2u) - (1 + s)^2}\right). \end{aligned} \tag{S3.43b}$$

Since the wildtype dominates at all times (unless rescue has occurred), we can again approximate $p_{\text{est}}^{(AB)} = 2\max[(s_{AB} - r), 0]$.

Fig. S3.3 shows P_{rescue} for various values of s_{ab} with all other parameter values as in Fig. 3C.

S3.4 Both single mutants have fitness greater than one

We here formalize the special case $s_{ab} = -1$, $s_{Ab} = s_{aB} = s > 0$. For this, we consider pairs consisting out of one Ab and one aB mutant. Such a pair reproduces at rate $\frac{1}{2} + \hat{s}$ and dies at rate $\frac{1}{2} - \hat{s}$ with $\hat{s} = \ln(1 + s)$. At rate $\frac{r}{2}(1 + s_{AB})$, it turns into an individual of type AB (this

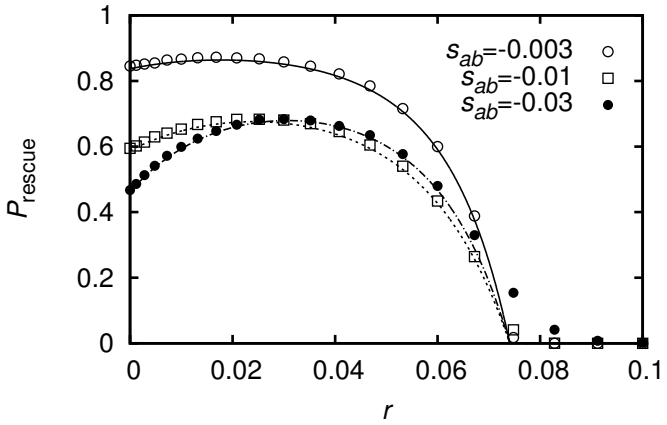

Fig. S3.3: **Probability of evolutionary rescue as a function of recombination for various values of s_{ab}.** All other parameter values are chosen as in Fig. 3C. Theoretical predictions are based on Eq. (S3.38) with Eq. (S3.43a). Symbols denote simulation results. Each simulation point is the average of $5 \cdot 10^4$ replicates. For the simulations with $s_{ab} = -0.003$, we considered a population as rescued when the number of double mutants reached $0.2N_0$ (changing the criterion to $0.3N_0$ did not alter the results).

ignores mutation). The growth rate of a pair is $2\hat{s}$, since in reality, we are not interested in pairs but establishment of any type (Ab, aB, AB) is fine, and each single mutant has growth rate s. However, it is pairs that convert into double mutants, and with this approximation, we assume that for every single mutant of type Ab, there is a single mutant of type aB to recombine with and vice versa. A single individual of type AB establishes a permanent lineage with probability $p_{\text{est}}^{(AB)} \approx 2s_{AB}$. Using Eq. (S1.8), we can calculate the survival probability of a process founded by exactly one pair:

$$\begin{aligned} p_{\text{est}}^{(Ab,aB)} &= 1 - \frac{1 + \hat{s} + \frac{r}{2}(1 + s_{AB}) - \sqrt{(\hat{s} - \frac{r}{2}(1 + s_{AB})^2 + (1 + 2\hat{s})r(1 + s_{AB})p_{\text{est}}^{(AB)}}}{1 + 2\hat{s}} \\ &\approx 2s - \frac{r}{2} + \sqrt{\left(2s - \frac{r}{2}\right)^2 + 2s_{AB}r}. \end{aligned} \tag{S3.44}$$

The probability of evolutionary rescue from generation 1 on is given by

$$1 - e^{-\bar{n}_{Ab}(1+s_{Ab})p_{\text{est}}^{(Ab,aB)}}. \tag{S3.45}$$

Neglecting the contribution of double mutants from the standing genetic variation to rescue, the possibility to generate the double mutant has a significant effect if either

$$p_{\text{est}}^{(Ab,aB)} \gg 4s \quad \text{or} \quad p_{\text{est}}^{(Ab,aB)} \ll 4s. \tag{S3.46}$$

These conditions simplify in few steps to

$$s_{AB} \gg 2s \quad \text{or} \quad s_{AB} \ll 2s. \tag{S3.47}$$

S3.5 Two-step rescue vs single-step rescue

We briefly discuss some instances where two-step rescue (as analyzed in this paper) is more likely to happen than single-step rescue (where there are only two types – the wildtype and the rescue type – and a single mutational step between them). For easier comparison, we denote the wildtype by ab and the rescue genotype by AB for single-step rescue as well. Mutation from wildtype to rescue mutants may happen with probability u_s. With Eq. (S1.4), the p.g.f. for the number of rescue mutations in the standing genetic variation is derived to be

$$F_{AB}^{\text{ssr}}(y) = \left(\frac{2\sigma_{AB}}{y + \sigma_{AB}y + \sigma_{AB} - 1}\right)^{2u_sN_0}. \tag{S3.48}$$

The probability of evolutionary rescue for single-step rescue is given by

$$\begin{aligned} P_{\text{rescue}}^{\text{ssr}} &= 1 - F_{AB}^{\text{ssr}}(e^{-(1+s_{AB})p_{\text{est}}^{(\text{AB})}})e^{-\frac{u_sN_0}{-s_{ab}}(1+s_{AB})p_{\text{est}}^{(AB)}} \\ &= 1 - e^{-p_{\text{est}}^{(AB)}(1+s_{AB})\left[\frac{u_sN_0}{-\sigma_{AB}}(1+\sigma_{AB})-\frac{u_sN_0}{-s_{ab}}\right]} \approx 1 - e^{-2s_{AB}\left[\frac{u_sN_0}{-\sigma_{AB}}-\frac{u_sN_0}{-s_{ab}}\right]}. \end{aligned} \tag{S3.49}$$

where the first summand in the brackets accounts for the contribution of standing genetic variation and the second one for new mutations after the environmental change (cf. also Orr and Unckless (2008, 2014); Bell and Collins (2008); Uecker *et al.* (2014)).

In the following, we focus on scenarios where the wildtype is lethal in the new environment and approximate single-step rescue by

$$P_{\text{rescue}}^{\text{sgv}} \approx 1 - e^{-2s_{AB}\frac{u_sN_0}{-\sigma_{AB}}}. \tag{S3.50}$$

Lethal single mutants. For two-step rescue, we use approximation Eq. (8):

$$P_{\text{rescue}} \approx 1 - e^{-2s_{AB}\frac{u^2N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1}} \overset{r \text{ large}/E_1=0}{\approx} 1 - e^{-2s_{AB}\frac{u^2N_0}{\sigma^2}}. \tag{S3.51}$$

Comparing with Eq. (S3.50) shows that two-step rescue is more likely if

$$\frac{u^2}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} > \frac{u_s}{-\sigma_{AB}}. \tag{S3.52}$$

For large recombination, this reduces to

$$\frac{u^2}{\sigma^2} > \frac{u_s}{-\sigma_{AB}}. \tag{S3.53}$$

For $E_1 = 0$ (which implies $\sigma_{AB} \approx 2\sigma$):

$$\frac{u^2}{-\sigma} > \frac{u_s}{2}. \tag{S3.54}$$

One viable single mutant. Following section S3.2, two-step rescue can be approximated by

$$1 - e^{-2s_{AB}\frac{u^2 N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1}} \times e^{-\frac{uN_0}{-\sigma}\frac{2s_{AB}u}{-s_{Ab}}}. \tag{S3.55}$$

Under these conditions, two-step rescue is more likely than single-step rescue if

$$\frac{u^2}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} + \frac{u^2}{\sigma s_{Ab}} > \frac{u_s}{-\sigma_{AB}}. \tag{S3.56}$$

Again, for strong recombination:

$$\frac{u^2}{\sigma^2} + \frac{u^2}{\sigma s_{Ab}} > \frac{u_s}{-\sigma_{AB}}. \tag{S3.57}$$

And for $E_1 = 0$:

$$\frac{u^2}{-\sigma} + \frac{u^2}{-s_{Ab}} > \frac{u_s}{2}. \tag{S3.58}$$

Viable single mutants. Last, we consider a scenario with both single mutants viable. With Eq. (10), the probability of evolutionary rescue is given by

$$1 - e^{-2s_{AB}\frac{u^2 N_0}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1}} \times e^{-2s_{AB}\frac{uN_0}{-\sigma}(1+s_{Ab})\frac{r}{r-2s_{Ab}}}. \tag{S3.59}$$

This yields for the condition that two-step rescue is more likely than single-step rescue

$$\frac{u^2}{\sigma^2}\frac{r-2\sigma}{r-2\sigma-E_1} + (1+s_{Ab})\frac{u}{-\sigma}\frac{r}{r-2s_{Ab}} > \frac{u_s}{-\sigma_{AB}}, \tag{S3.60}$$

which for strong recombination simplifies to

$$\frac{u}{-\sigma}\left(\frac{u}{-\sigma} + (1+s_{Ab})\frac{r}{r-2s_{Ab}}\right) > \frac{u_s}{-\sigma_{AB}}. \tag{S3.61}$$

For $E_1 = 0$:

$$\frac{u^2}{-\sigma} + (1+s_{Ab})\frac{ur}{r-2s_{Ab}} > \frac{u_s}{2}. \tag{S3.62}$$

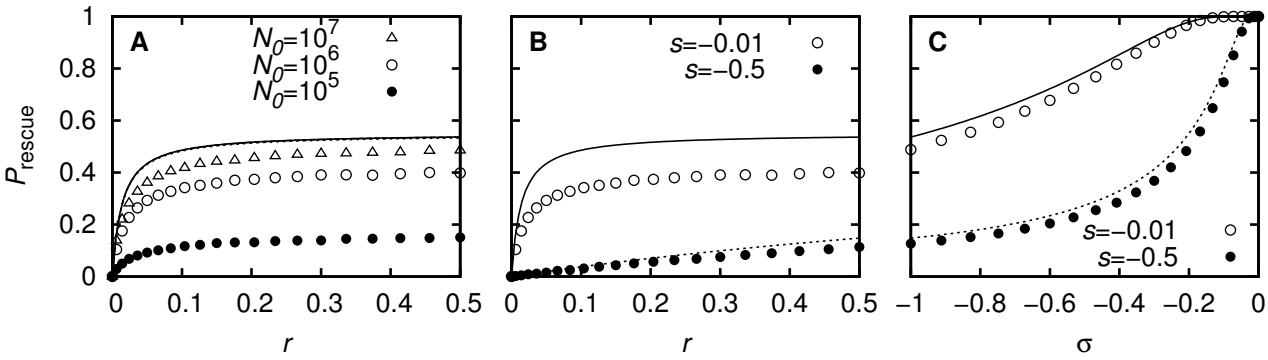

Fig. S4.4: **Probability of evolutionary rescue as a function of recombination for various population sizes N_0 with $N_0 s_{AB} = 2000$ kept constant (Panel A), recombination r for various values of s (Panel B), and the strength of selection against single mutants in the old environment σ (Panel C).** The figure varies parameters from Fig. 3A. For all Panels: $u = 2 \cdot 10^{-6}$, $\sigma_{AB} = -0.1$, $s_{ab} = -1$. Panel A: $N_0 s_{AB} = 2000$, $\sigma = -0.01$, $s = -0.01$; Panel B: $s_{AB} = 0.002$, $\sigma = -0.01$, $N_0 = 10^6$; Panel C: $s_{AB} = 0.002$, $r = 0.5$, $N_0 = 10^8$. Symbols denote simulation results. Each simulation point is the average of $5 \cdot 10^4$ replicates.

File S4: Limits of the approximations

Our approximations assume that wildtype individuals and single mutants are sufficiently frequent to describe their dynamics deterministically. This requires a sufficiently large population size and a sufficiently high fitness of single mutants prior to the change in the environment. Fig. S4.4 takes Fig. 3A as a starting point and varies several parameters in order to probe the limits of the approximations. Panel A shows P_{rescue} for various initial population sizes N_0 with the product $N_0 s_{AB}$ kept constant such that the theoretical predictions virtually coincide. However, as the population size gets smaller, simulation results greatly deviate from this prediction. Note that the number of single mutants for $N_0 = 10^5$ is as low as $\bar{n}_{Ab} = \bar{n}_{aB} = 20$. While in Panel A the number of single mutants in the standing genetic variation differs for different population sizes, it is – on average – the same at the right edge of Panel B ($N_0 = 10^6$, $\sigma = -0.01$) and the left edge of Panel C ($N_0 = 10^8$, $\sigma = -1$) but stochasticity is higher in Panel B, leading to larger deviations between the analytical prediction and simulation results.

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