Supporting information – Evolutionary rescue and drug resistance on multicopy plasmids

S1: Alternative models of plasmid replication and segregation

In this section, we present two alternative models of plasmid replication and segregation. For the model described in the main text, we assume that prior to cell division each plasmid is replicated once and all copies are randomly distributed to the daughter cells, where each daughter cell reveives n copies. For the alternative models, we assume that at cell division plasmids are distributed to both daughter cells *before* they replicate to reach their copy number. We consider two variants: (1) each cell receives $n/2$ plasmid copies for n even or $(n-1)/2$ and $(n+1)/2$ if n is uneven (2) each daughter cell receives at least one plasmid; the remaining plasmids are randomly distributed to the daughter cells such that in general, daughter cells receive different numbers of plasmid copies. Subsequently, plasmids get replicated one plasmid at a time until there are n plasmid copies in the cell. The plasmid copy that is replicated is chosen randomly from all plasmids in the daughter cell (including those that have just been generated). Therefore, the plasmid composition changes during the plasmid replication phase. Moreover, an early mutation appearing during the replication phase can immediately lead to a daughter cell with a number of mutated plasmids greater than 1. We assume that plasmid replication occurs immediately after cell division, and birth and death rates of cells depend on their final plasmid composition. All results for the alternative models were obtained by stochastic computer simulations.

Fig. S1.1 shows the probabilities of *de novo* rescue under the two alternative models for the same parameter combinations as Fig. 2. The results obtained with the two variants of the alternative model are very similar to each other. In Fig. S1.2, we directly compare the results from the alternative models to the original model from the main text. While the general trends mostly remain the same, some differences arise. The most prominent deviations arise for recessive mutations. Under the original model, the rescue probability drops very quickly with increasing plasmid copy number. With the alternative schemes

of plasmid replication and segregation, a high copy number has a less negative effect on rescue, and there is even a slight maximum for low copy numbers. Likewise, for mutations of intermediate dominance, the negative effect of high copy numbers is also weaker. In that case, this makes that we do not observe the slight intermediate maximum anymore that we observed under the original model.
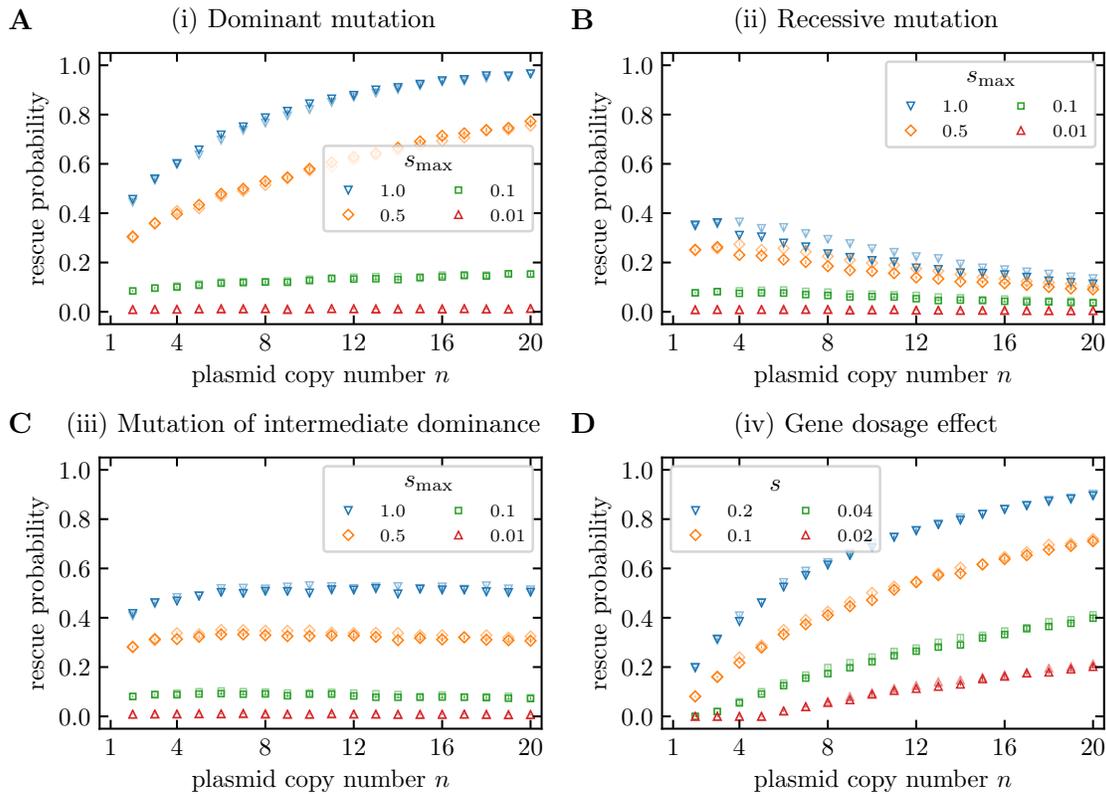

Figure S1.1: **Rescue probabilities under two alternative schemes of plasmid replication and segregation.** For both schemes, plasmids are at first segregated into the daughter cells, and subsequently plasmids get replicated one by one until the cell contains n plasmid copies. For alternative 1 (dark colored markers), plasmid are segregated to the daughter cells in equal numbers $n/2$ if n is even and one daughter cell receives one more plasmid than the other if n is uneven. For alternative 2 (light colored markers), each daughter cell receives at least one plasmid copy, and the remaining copies are segregated randomly. The parameters correspond to those in Fig. 2A-D in the main text. Results were obtained by 10^4 stochastic simulations (10^5 for alternative 1 and $n \leq 10$). Error bars indicate standard errors.
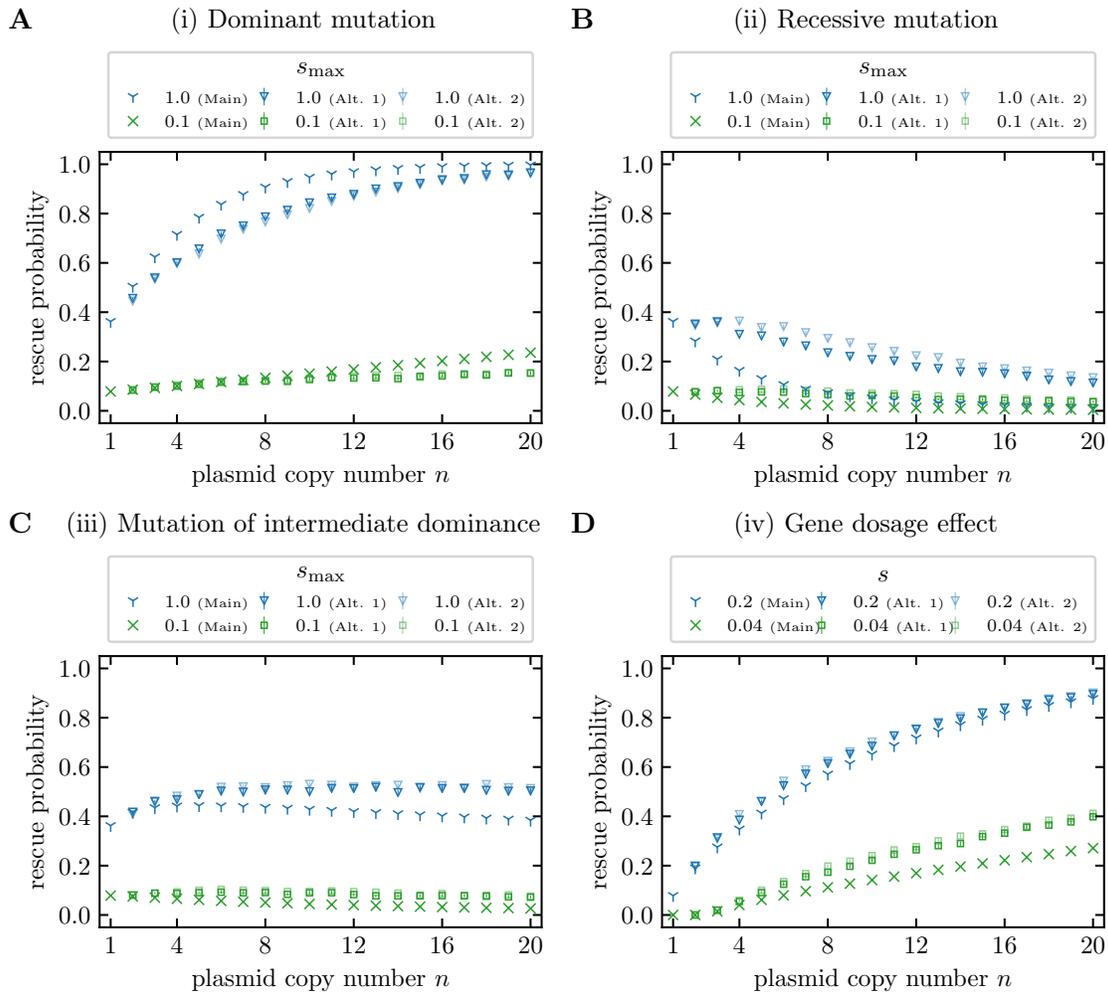
Figure S1.2: **Comparison of rescue probabilities from the main text model with results obtained from the alternative models.** The alternative models are described in the text and in the caption of Fig. S1.1. As a reminder, for alternative 1 (dark open colored markers), plasmid are segregated to the daughter cells in equal numbers $n/2$ if n is even and one daughter cell receives one more plasmid than the other if n is uneven. For alternative 2 (light open colored markers), each daughter cell receives at least one plasmid copy, and the remaining copies are segregated randomly. The parameters correspond to those in Fig. 2A-D in the main text and in Fig. S1.1 in the SI. Results for the main text model were obtained numerically by using Eq. (3) with Eq. (A.5) and Eq. (A.1). For the alternative models, results were obtained by 10^4 stochastic simulations (10^5 for alternative 1 and $n \leq 10$). Error bars indicate standard errors.

S2: Analytical derivations for plasmid copy numbers $n = 1$ and $n = 2$

S2.1 Establishment probabilities

We here outline the derivation of the analytical solution for $p_{\text{est}}^{(n=2)}$ given by Eq. (5) from the general Equation (A.2). For $n = 2$, there are three cell-types $i = 0$, $i = 1$, and $i = 2$ and we get a coupled system of equations from Eq. (A.2) with $i = 1$ and $i = 2$ for the extinction probabilities $Q_i^{(2)}$ for a lineage founded by a cell of type i:

$$Q_1^{(2)} = \frac{\mu_1^{(2)}}{\lambda_1^{(2)} + \mu_1^{(2)}} + \frac{\lambda_1^{(2)}}{\lambda_1^{(2)} + \mu_1^{(2)}} \sum_{k=0}^{1} P(1 \to \{k, 2-k\}) Q_k^{(2)} Q_{2-k}^{(2)}$$

$$= \frac{\mu_1^{(2)}}{\lambda_1^{(2)} + \mu_1^{(2)}} + \frac{\lambda_1^{(2)}}{\lambda_1^{(2)} + \mu_1^{(2)}} \left(\frac{1}{3} Q_2^{(2)} + \frac{2}{3} Q_1^{(2)^2} \right), \tag{S2.1}$$

$$Q_2^{(2)} = \frac{\mu_2^{(2)}}{\lambda_2^{(2)} + \mu_2^{(2)}} + \frac{\lambda_2^{(2)}}{\lambda_2^{(2)} + \mu_2^{(2)}} Q_2^{(2)} \tag{S2.2}$$

where we have used that $Q_0^{(2)} = 1$ (see main text). By inserting the solution $Q_2^{(2)} = \frac{\mu_2^{(2)}}{\lambda_2^{(2)}}$ of Equation (S2.2) into Eq. (S2.1) and solving this equation, we obtain the solutions x_1 and x_2 for $Q_1^{(2)}$:

$$x_{1,2} = \frac{3}{4} + \frac{3}{4} \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} \pm \frac{1}{4} \sqrt{9 - 6 \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} + 9 \left(\frac{\mu_1^{(2)}}{\lambda_1^{(2)}} \right)^2 - 8 \frac{\mu_2^{(2)}}{\lambda_2^{(2)}}}. \tag{S2.3}$$

Those solutions are both real numbers since $9 - 6 \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} + 9 \left(\frac{\mu_1^{(2)}}{\lambda_1^{(2)}} \right)^2 - 8 \frac{\mu_2^{(2)}}{\lambda_2^{(2)}} = 8 \left(1 - \frac{\mu_2^{(2)}}{\lambda_2^{(2)}} \right) + \left(1 - 3 \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} \right)^2 > 0$ (remember that $\mu_2^{(2)} < \lambda_2^{(2)}$). For the solution x_1, it holds that

$$\frac{3}{4} + \frac{3}{4} \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} + \frac{1}{4} \sqrt{8 \left(1 - \frac{\mu_2^{(2)}}{\lambda_2^{(2)}} \right) + \left(1 - 3 \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} \right)^2} \tag{S2.4}$$

$$> \frac{3}{4} + \frac{3}{4} \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} + \frac{1}{4} \sqrt{\left(1 - 3 \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} \right)^2} \tag{S2.5}$$

$$= \frac{3}{4} + \frac{3}{4} \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} + \frac{1}{4} \left| 1 - 3 \frac{\mu_1^{(2)}}{\lambda_1^{(2)}} \right| > 1. \tag{S2.6}$$

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Therefore, the other solution, x_2, gives the extinction probability $Q_1^{(2)} = x_2$. The establishment probability is given by $p_{\text{est}}^{(n=2)} = 1 - Q_1^{(2)}$ (see Eq. (5)).

Next, we derive inequality (9) that tells when the establishment probability is higher for $n = 2$ than for $n = 1$ in scenario 2 (gene dosage effects). Birth rates $\lambda_i^{(n)}$ and death rates $\mu_i^{(n)}$ depend on the number of mutated plasmids i in this scenario. Therefore we use birth rates λ_1, λ_2 and death rates μ_1, μ_2 independent of the plasmid copy number n as defined in the main text.

The establishment probabilities for $n = 2$ is higher compared to $n = 1$ if $p_{\text{est}}^{(n=2)} > p_{\text{est}}^{(n=1)}$. Insertion of the solutions for the establishment probabilities (Eq. (4) and (5)) gives

$$\frac{1}{4} - \frac{3}{4}\frac{\mu_1}{\lambda_1} + \frac{1}{4}\sqrt{9 - 6\frac{\mu_1}{\lambda_1} + 9\left(\frac{\mu_1}{\lambda_1}\right)^2 - 8\frac{\mu_2}{\lambda_2}} > 1 - \frac{\mu_1}{\lambda_1}$$

$$\Leftrightarrow \quad \frac{1}{4}\sqrt{9 - 6\frac{\mu_1}{\lambda_1} + 9\left(\frac{\mu_1}{\lambda_1}\right)^2 - 8\frac{\mu_2}{\lambda_2}} > \frac{3}{4} - \frac{1}{4}\frac{\mu_1}{\lambda_1}$$

$$\Leftrightarrow \quad 9 - 6\frac{\mu_1}{\lambda_1} + 9\left(\frac{\mu_1}{\lambda_1}\right)^2 - 8\frac{\mu_2}{\lambda_2} > \left(3 - \frac{\mu_1}{\lambda_1}\right)^2 \tag{S2.7}$$

$$\Leftrightarrow \quad \left(\frac{\mu_1}{\lambda_1}\right)^2 > \frac{\mu_2}{\lambda_2}$$

$$\Leftrightarrow \quad \frac{\lambda_2}{\mu_2} > \left(\frac{\lambda_1}{\mu_1}\right)^2 .$$

where we used $\mu_1 < \lambda_1$ and hence $3 - \mu_1/\lambda_1 > 0$ from line two to line three (otherwise, rescue would not be possible for $n = 1$).

S2.2 Rescue probabilities

We first derive the inequality (6) which tells when rescue is more likely for $n = 2$ than for $n = 1$ in scenario 1 (no gene dosage effects). As a reminder, for scenario 1 we defined $\lambda_{\mathsf{hom}} = \lambda_1^{(1)} = \lambda_2^{(2)}$, $\mu_{\mathsf{hom}} = \mu_1^{(1)} = \mu_2^{(2)}$ and $\lambda_{\mathsf{het}} = \lambda_1^{(2)}$, $\mu_{\mathsf{het}} = \mu_1^{(2)}$. From Eq. (3) we know that rescue is more likely to occur on a two-copy plasmid if and only if

$$
\begin{aligned}
& 1 - e^{-2u\lambda_0 \frac{N_0}{\mu_0 - \lambda_0} p_{\mathrm{est}}^{(n=2)}} > 1 - e^{-u\lambda_0 \frac{N_0}{\mu_0 - \lambda_0} p_{\mathrm{est}}^{(n=1)}} \\
\Leftrightarrow\quad & 2 p_{\mathrm{est}}^{(n=2)} > p_{\mathrm{est}}^{(n=1)} \\
\Leftrightarrow\quad & 2 \left(\frac{1}{4} - \frac{3}{4} \frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} + \frac{1}{4} \sqrt{9 - 6\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} + 9 \left(\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} \right)^2 - 8\frac{\mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}}} \right) > \frac{\lambda_{\mathrm{hom}} - \mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}} \\
\Leftrightarrow\quad & \sqrt{9 - 6\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} + 9 \left(\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} \right)^2 - 8\frac{\mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}}} > 2\frac{\lambda_{\mathrm{hom}} - \mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}} + 3\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} - 1 \\
\Leftrightarrow\quad & 9 - 6\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} + 9 \left(\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} \right)^2 - 8\frac{\mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}} > \left(2\frac{\lambda_{\mathrm{hom}} - \mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}} + 3\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} - 1 \right)^2 \\
\overset{(\ldots)}{\Leftrightarrow}\quad & \frac{\lambda_{\mathrm{het}}}{\mu_{\mathrm{het}}} > \frac{3\frac{\lambda_{\mathrm{hom}}}{\mu_{\mathrm{hom}}}}{1 + 2\frac{\lambda_{\mathrm{hom}}}{\mu_{\mathrm{hom}}}},
\end{aligned}
\tag{S2.8}
$$

where we used from line four to line five that

$$
\begin{aligned}
& 2\frac{\lambda_{\mathrm{hom}} - \mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}} + 3\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} - 1 \\
=\; & 1 - \frac{\mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}} + 3\frac{\mu_{\mathrm{het}}}{\lambda_{\mathrm{het}}} - 2\frac{\mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}} \\
\geq\; & 1 - \frac{\mu_{\mathrm{hom}}}{\lambda_{\mathrm{hom}}} + 3\frac{\mu_{\mathrm{hom}}}{\lambda_{\mathrm{het}}} - 2\frac{\mu_{\mathrm{hom}}}{\lambda_{\mathrm{het}}} > 0,
\end{aligned}
$$

where, in turn, we used $\mu_{\mathrm{hom}} < \lambda_{\mathrm{hom}}$ and our model assumptions $\mu_{\mathrm{het}} \geq \mu_{\mathrm{hom}}$ and $\lambda_{\mathrm{het}} \leq \lambda_{\mathrm{hom}}$.

Last, we provide the mathematical proof for the statement (made in the main text) that the probability of evolutionary rescue P_{rescue} is higher for $n = 2$ plasmids per cell compared to $n = 1$ in a scenario in scenario 2 (gene dosage effects). As in the main text, we denote birth and death rates by λ_0, λ_1, λ_2 and death rates μ_0, μ_1, μ_2. Furthermore, we define the ratio of death and birth rates $\rho_1 \equiv \frac{\mu_1}{\lambda_1}$ and $\rho_2 \equiv \frac{\mu_2}{\lambda_2}$.

It needs to be shown that the rescue probability given by Eq. (3) for $n = 2$ is higher than for $n = 1$. This is equivalent to

$$
1 - e^{-2u\lambda_0 \frac{N_0}{\mu_0 - \lambda_0} p_{\mathrm{est}}^{(2)}} > 1 - e^{-u\lambda_0 \frac{N_0}{\mu_0 - \lambda_0} p_{\mathrm{est}}^{(1)}},
$$

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which can be transformed into the following inequality:

$$2p_{\text{est}}^{(2)} > p_{\text{est}}^{(1)}. \tag{S2.9}$$

In the 'worst' case, the second plasmid brings no additional benefit, i.e. $\rho_2 = \rho_1 \equiv \rho < 1$. In this scenario, establishment probabilities for one and two plasmids per cell (see Eq. (4)) become

$$p_{\text{est}}^{(n=1)} = 1 - \rho,$$
$$p_{\text{est}}^{(n=2)} = \frac{1}{4} - \frac{3}{4}\rho + \frac{1}{4}\sqrt{9 - 14\rho + 9\rho^2}.$$

Inserting the establishment probabilities in Eq. (S2.9) shows that the statement is correct:

$$\frac{1}{2} - \frac{3}{2}\rho + \frac{1}{2}\sqrt{9 - 14\rho + 9\rho^2} > 1 - \rho$$
$$\Leftrightarrow \quad \sqrt{9 - 14\rho + 9\rho^2} > 1 + \rho$$
$$\Leftrightarrow \quad 9 - 14\rho + 9\rho^2 > 1 + 2\rho + \rho^2$$
$$\Leftrightarrow \quad 1 - 2\rho + \rho^2 > 0$$
$$\Leftrightarrow \quad (1 - \rho)^2 > 0.$$

S3: Evolutionary rescue by new mutations

## (i) Dominant mutation
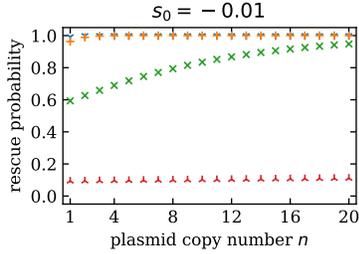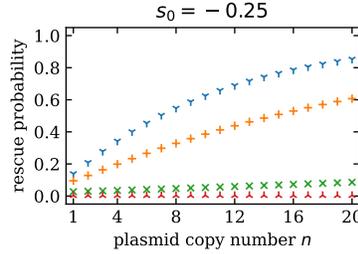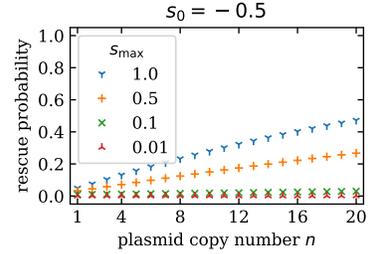
(ii) Recessive mutation
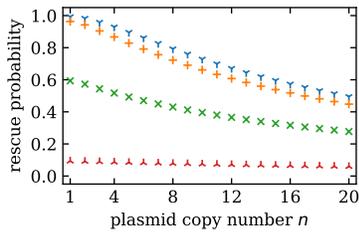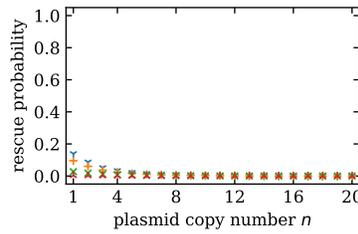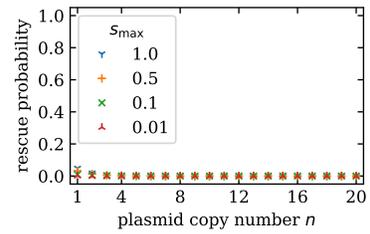
## (iii) Mutation of intermediate dominance
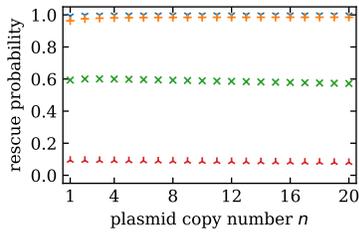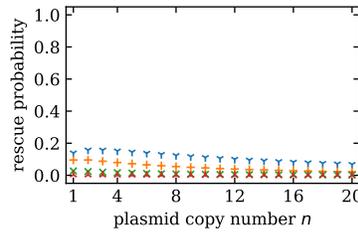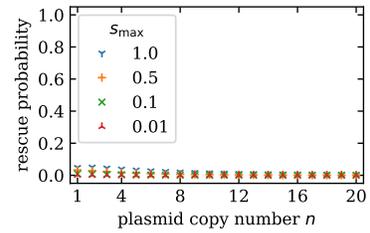
## (iv) Gene dosage effect
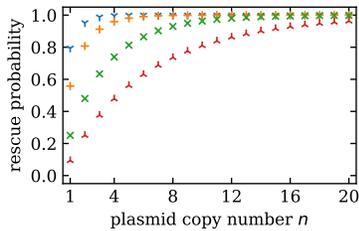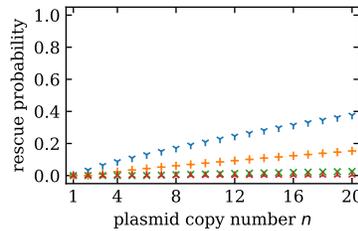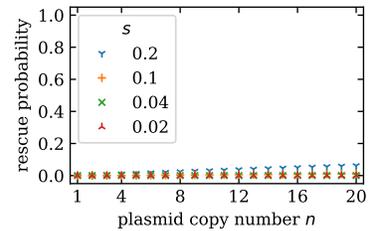
Figure S3.1: **Probabilities of evolutionary rescue from *de novo* mutations.** The columns correspond to the right column of Fig. 2 in the main text with different fitness $\lambda_0^{(n)} = 1 + s_0$ of wild-type homozygotes. In Panel C, the blue and orange curves slightly decrease again for even larger plasmid copy numbers that are outside the range of the plot (For $s_{\max} = 0.5$, we obtain: $P_{\text{rescue}}^{(n=1)} = 96.3$, $P_{\text{rescue}}^{(n=20)} = 98.5$, $P_{\text{rescue}}^{(n=50)} = 98.4$, $P_{\text{rescue}}^{(n=100)} = 98.1$. For $s_{\max} = 1$, we obtain: $P_{\text{rescue}}^{(n=1)} = 99.292$, $P_{\text{rescue}}^{(n=20)} = 99.972$, $P_{\text{rescue}}^{(n=50)} = 99.975$, $P_{\text{rescue}}^{(n=100)} = 99.972$.).

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S4: The effect of the variance in the cell type numbers in the standing genetic variation

Fig. S4.1 shows the probability of rescue from the standing genetic variation with all parameters being equal as in Fig. 5 except for that the population size is smaller by an order of magnitude. Deviations between the analytical theory and the stochastic simulations are larger for smaller populations.
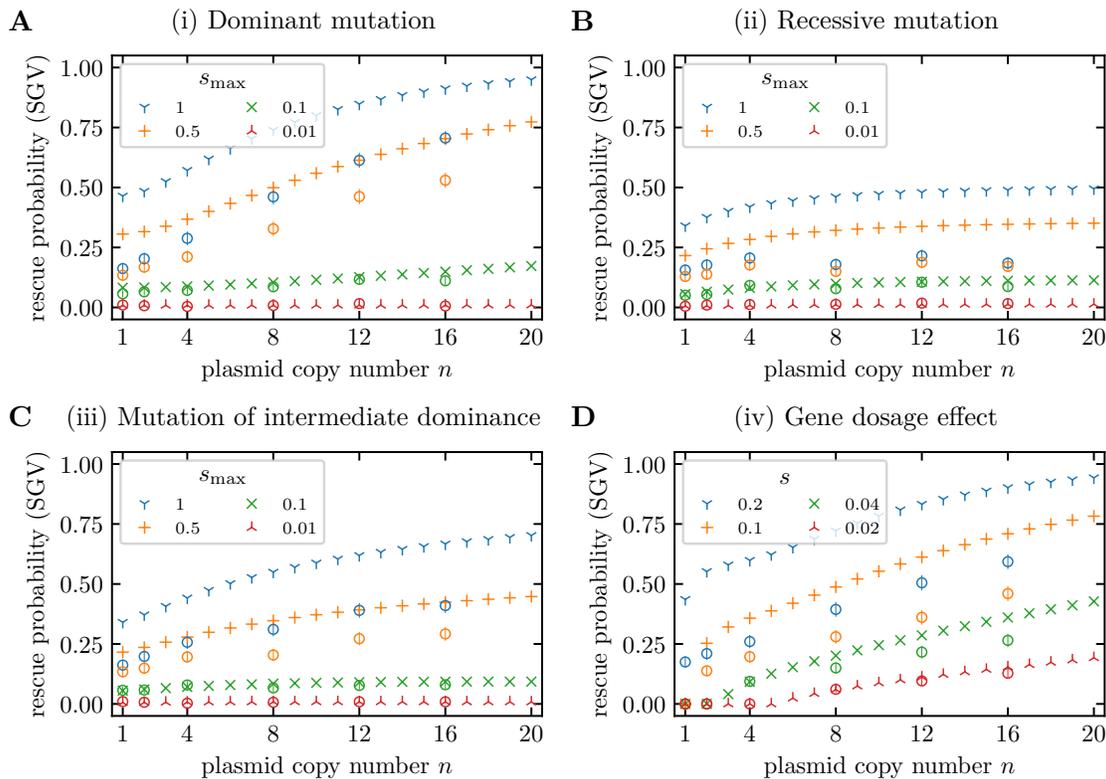

Figure S4.1: **Probabilities of evolutionary rescue from standing genetic variation.** The figure corresponds to Fig. 5 in the main text except for a smaller population size of $N_0 = 3 \times 10^8$ compared to $N = 3 \times 10^9$ in Fig. 5. Closed markers show the results obtained by deterministic frequencies of mutant cells in the standing genetic variation. Open markers show results from 10^3 independent stochastic simulations.

In the main text, we hypothesized that the decrease in $P_{\text{rescue}}^{(\text{SGV})}$ for large n for recessive mutations is caused by a higher variance in the number of cells N_i with high n. Here, we investigate this in a bit more detail. To this purpose, we choose to consider a scenario where the wildtype (and hence all heterozygous cells) are lethal in the new environment. Hence, rescue – if it occurs – occurs from mutant homozygotes in the standing genetic variation.
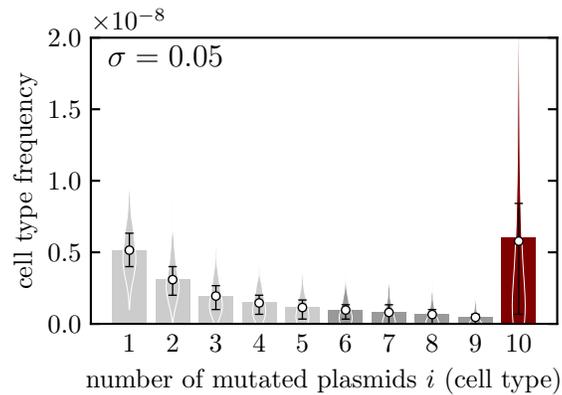
Figure S4.2: **Cell type frequencies in the standing genetic variation.** The figure reproduces Fig. 4F with a larger range of the y-axis such that the entire error bar (interquartile range) for the frequency of the homozygous type (red) is visible.

Fig. S4.3A shows the frequency of mutant homozygotes in dependence of the plasmid copy number, and Panel B shows their variance. One can see that the mean frequency remains constant but the variance indeed increases with n. In line with $N_n = \text{const.}$, the analytical theory predicts that $P_{\text{rescue}}^{(\text{SGV})}$ does not change with n (Fig. S4.3). In contrast, the results obtained from stochastic computer simulations decline as n increases, which confirms our intuition.

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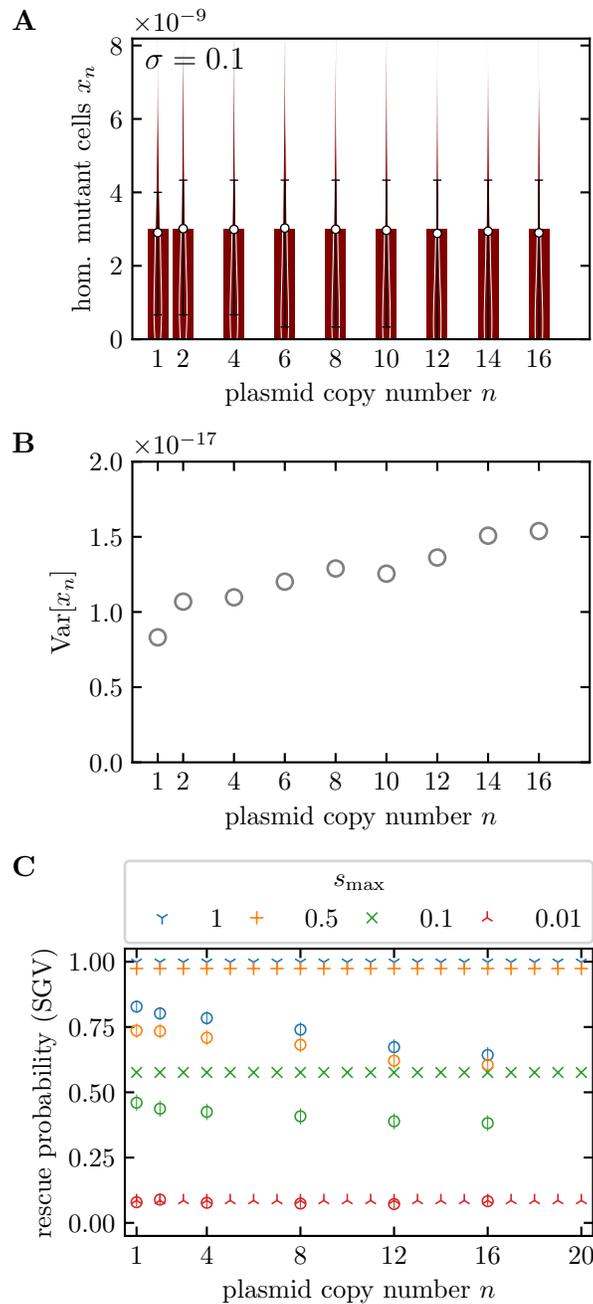
Figure S4.3: **Influence of stochasticity in the cell type frequencies in the standing genetic variation on the probability of evolutionary rescue.** Panel A: Frequencies of homozygous mutant cells $x_n = N_n/N$ for various plasmid copy numbers obtained from the deterministic mutation-selection equilibrium (bars) and from stochastic simulations (violin plots) for a recessive mutation with selection coefficient $-\sigma$. Open markers and error bars show the mean and the interquartile range of 10^3 stochastic simulations). Panel B: Variance $\mathrm{Var}[x_n] = \sigma_{x_n}^2$ of the number of homozygous mutant cells x_n obtained from 10^3 stochastic simulations (cf. error bars in Panel B). Panel C: Probability of evolutionary rescue from standing genetic variation considering a scenario where heterozygous cells (as well as wild-type cells) are lethal. Closed markers show the results obtained using deterministic frequencies of mutant cells in the standing genetic variation. Open markers show simulation results from 10^3 simulations. All parameters are the same as in Fig. 5 (main text) except for the birth rate of wild-type and heterozygous cells $\lambda_0^{(n)} = 0$ in the presence of antibiotics.