Supplementary Note on the Value r

The value of r in the mathematical model determines the extent to which the fitness advantage of a mutation biases the likelihood that it is the next population genotype to fix. Throughout our analysis we take $r = 0$, corresponding to a random walk on the fitness landscape wherein the next population genotype is chosen randomly amongst all neighbouring fitter genotypes. This value of r corresponds to the model proposed by Flyvbjerg and Lautrup [1], Macken and Perelson [6], Macken et al. [7].

An alternative model, arising from Gillespie [3, 4], is to take $r = 1$. Under such a model our results are qualitatively unchanged. Of the 82,944 unique tables of collateral response the most likely occurs with probability 0.0097 for $r = 1$ (0.0023 for $r = 0$). Amongst the 225 ordered drug pairs with $r = 1$ we still find only 28 with guaranteed collateral sensitivity, 94 with guaranteed cross resistance, 15 for which the first drug makes no difference, and 88 for which the first drug can induce either collateral sensitivity or cross resistance in the second. If a collateral response table is generated by stochastic *in silico* simulation, and a collaterally sensitive drug pair chosen at random, then the first of these two drugs will induce cross resistance in the second with expected probability 0.548 (0.513 for $r = 0$) as determined from 10^6 simulations of this process.

Note that as $r \to \infty$, the biased random walk becomes a deterministic walk in which the fittest neighbouring genotype always achieves fixation. In this case, evolution is always repeatable and the collateral response is stable. The model $r \to \infty$ has been proposed previously for protein evolution, for example by Fontana et al. [2], Kauffman and Levin [5], but is likely inappropriate for modelling evolution in asexually reproducing populations, as evidenced by our own observations of evolutionary divergence during experimental evolution.

Antibiotic	Abbreviation	Group	Concentration
Ampicillin	AMP	Aminopenicillin	2,048 μg/ml
Amoxicillin	AM	Aminopenicillin	512 μg/ml
Cefaclor	CEC	Cephalosporin	1 μg/ml
Cefotaxime	CTX	Cephalosporin	0.05 μg/ml
Ceftizoxime	ZOX	Cephalosporin	0.03 μg/ml
Cefuroxime	CXM	Cephalosporin	1.5 μg/ml
Ceftriaxone	CRO	Cephalosporin	0.045 μg/ml
Amoxicillin + clavulanic acid	AMC	Penicillin derivative + β-Lactamase inhibitor	512 μg/ml (amoxicillin) and 8 μg/ml (clav)
Ceftazidime	CAZ	Cephalosporin	0.1 μg/ml
Cefotetan	CTT	Cephalosporin	0.312 μg/ml
Ampicillin + sulbactam	SAM	Penicillin derivative + β-Lactamase inhibitor	8 μg/ml (ampicillin) and 8 μg/ml (sulbactam)
Cefprozil	CPR	Cephalosporin	100 μg/ml
Cefpodoxime	CPD	Cephalosporin	2 μg/ml
Pipercillin + tazobactam	TZP	Penicillin derivative + β-Lactamase inhibitor	12μg/ml (pipercillin) and 8 μg/ml (tazobactam)
Cefepime	FEP	Cephalosporin	0.0156 μg/ml

Table 1. 15 β–lactam antibiotics for which Mira et al (2015) derived fitness landscapes.

Supplementary Table 2

Table listing the MICs for evolutionary replicates X1-X12 following passage 10 for the panel of 40 antibiotic drugs. The drug name and class are indicated in addition to the MIC values. Note that, for expedience, MIC values are specified as the least power of two (in units of μg/ml) that inhibits growth. Inequalities indicate minimum or maximum values for the MIC in cases where the MIC value was not derived because the observed minimum/maximum values were sufficient to rule out divergent collateral response.

[Supplementary Table 2 is provided as a .xls spreadsheet]

Supplementary Figures

Below we present figures 9 figures showing the the full extent of non–repeatable evolution for the fifteen drugs. Each figure corresponds to five first–line and five second–line drugs and consists of 25 subplots, one for each pair of drugs. Points in these subplots represent accessible local optima genotypes in the fitness landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution (from $g_0 = 0000$) in the landscape of the labelled drug, as determined by the mathematical model (with $r = 0$, see Materials and Methods for details). The y value in each subplot corresponds to the fitness of that genotype in a second landscape (as labelled). The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

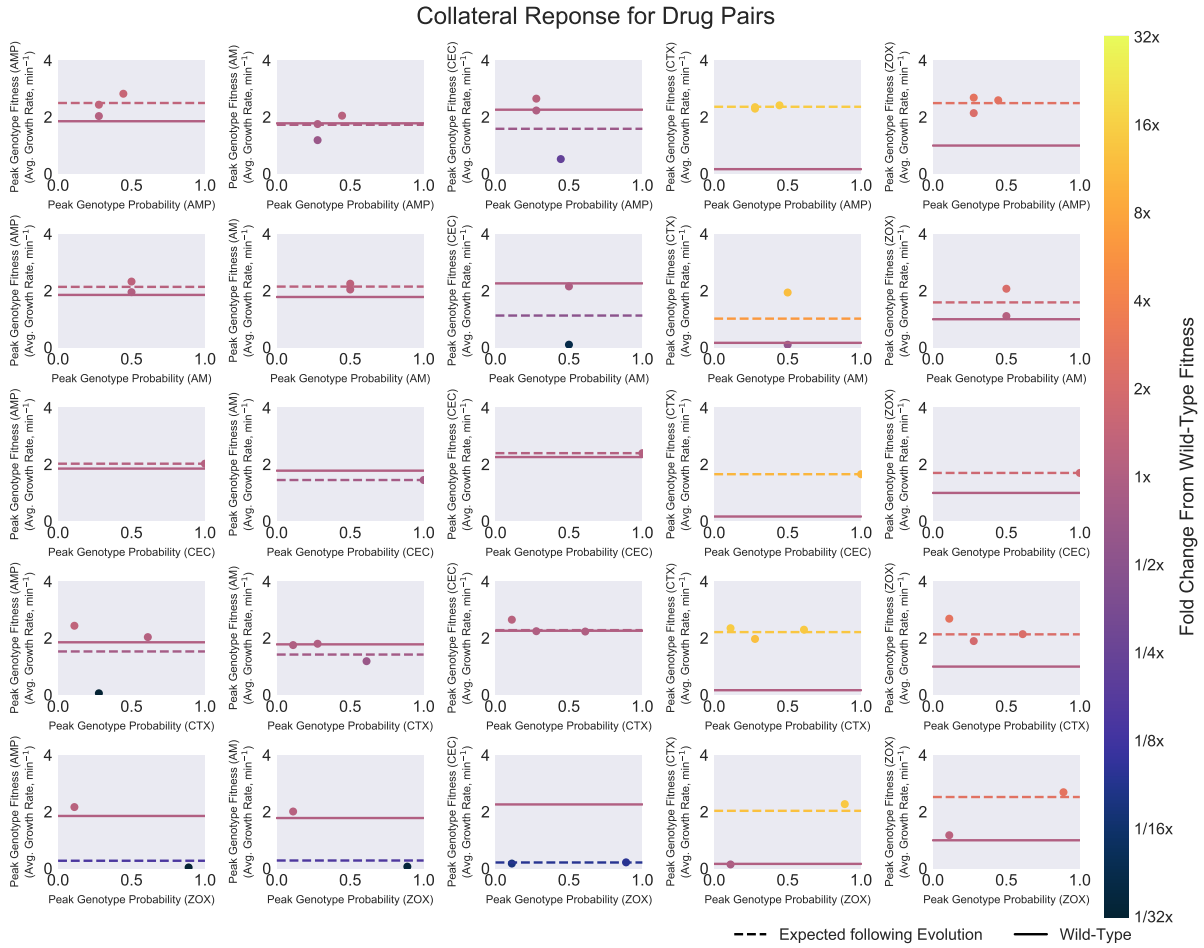

Figure 1. Collateral response between drugs in the mathematical model. Each subplot corresponds to a pair of drugs. Points represent accessible local optima genotypes in the landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution in the landscape of the labelled drug, and y value corresponding to the fitness of that genotype in the second landscape. The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

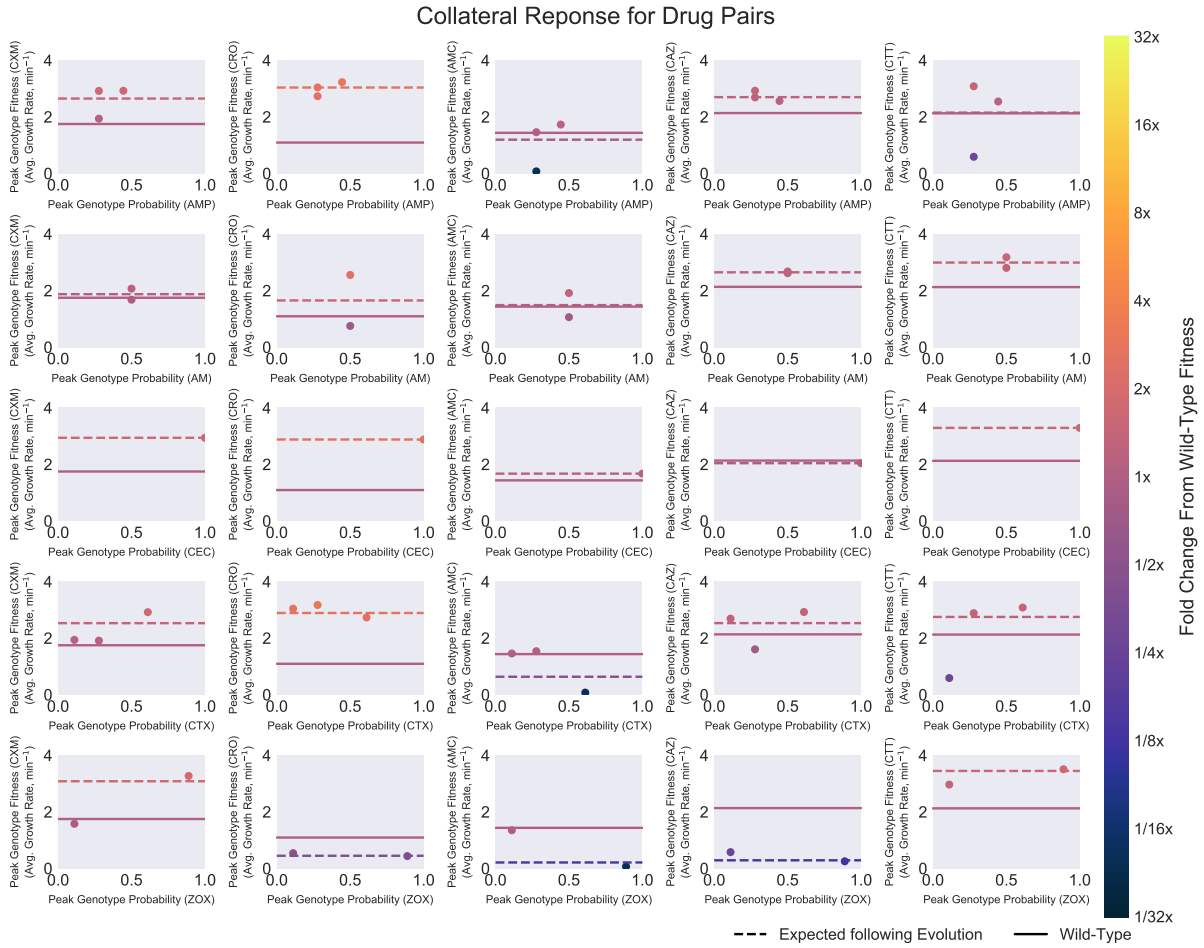

Figure 2. Collateral response between drugs in the mathematical model. Each subplot corresponds to a pair of drugs. Points represent accessible local optima genotypes in the landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution in the landscape of the labelled drug, and y value corresponding to the fitness of that genotype in the second landscape. The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

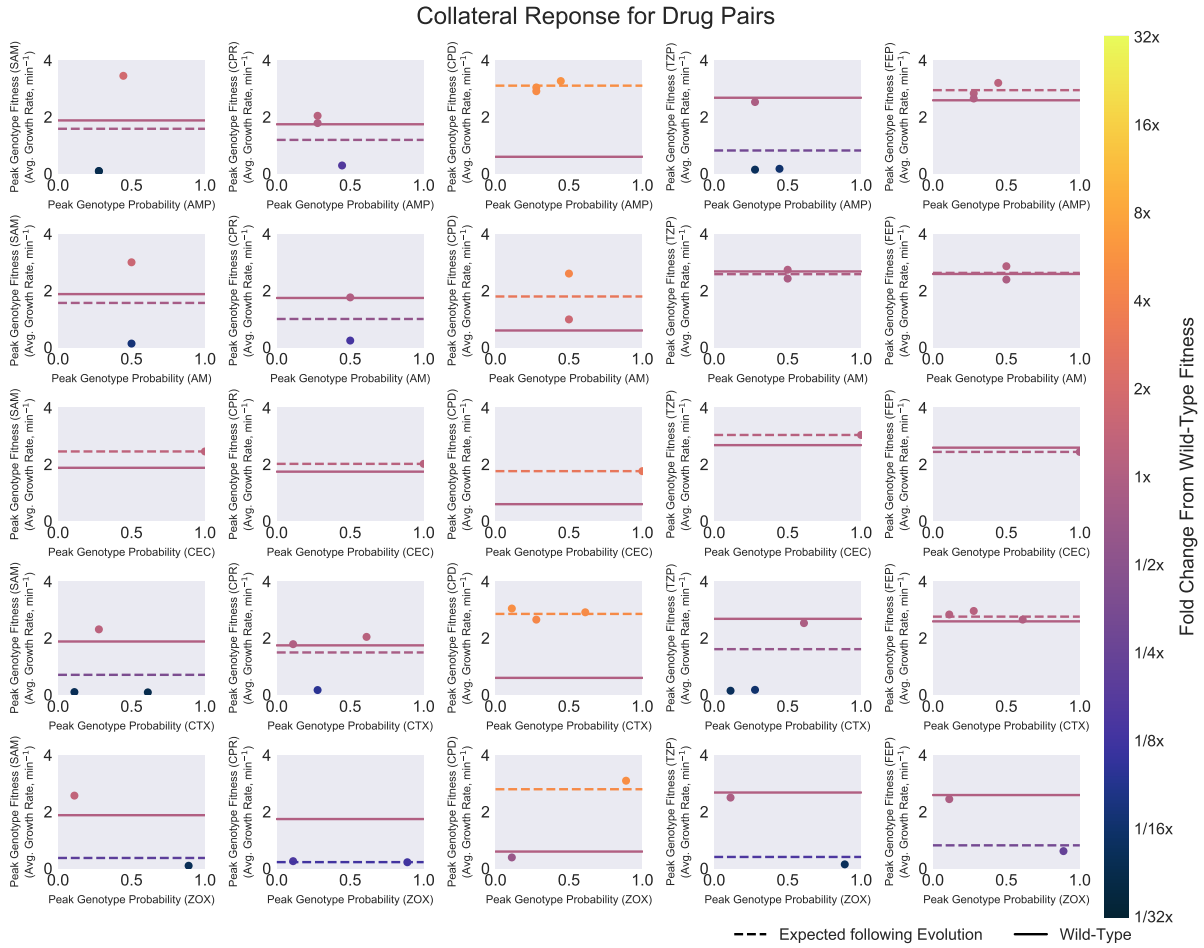

Figure 3. Collateral response between drugs in the mathematical model. Each subplot corresponds to a pair of drugs. Points represent accessible local optima genotypes in the landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution in the landscape of the labelled drug, and y value corresponding to the fitness of that genotype in the second landscape. The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

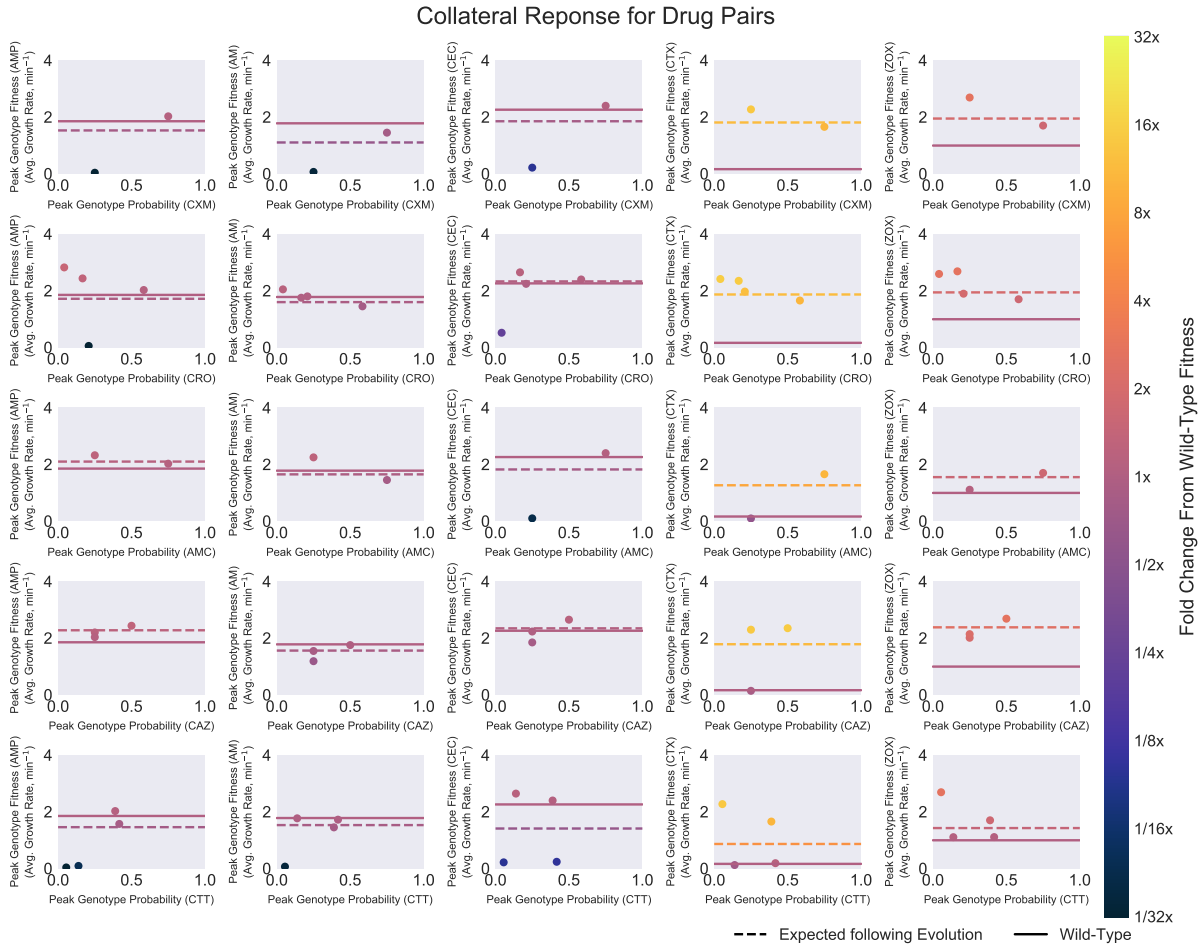

Figure 4. Collateral response between drugs in the mathematical model. Each subplot corresponds to a pair of drugs. Points represent accessible local optima genotypes in the landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution in the landscape of the labelled drug, and y value corresponding to the fitness of that genotype in the second landscape. The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

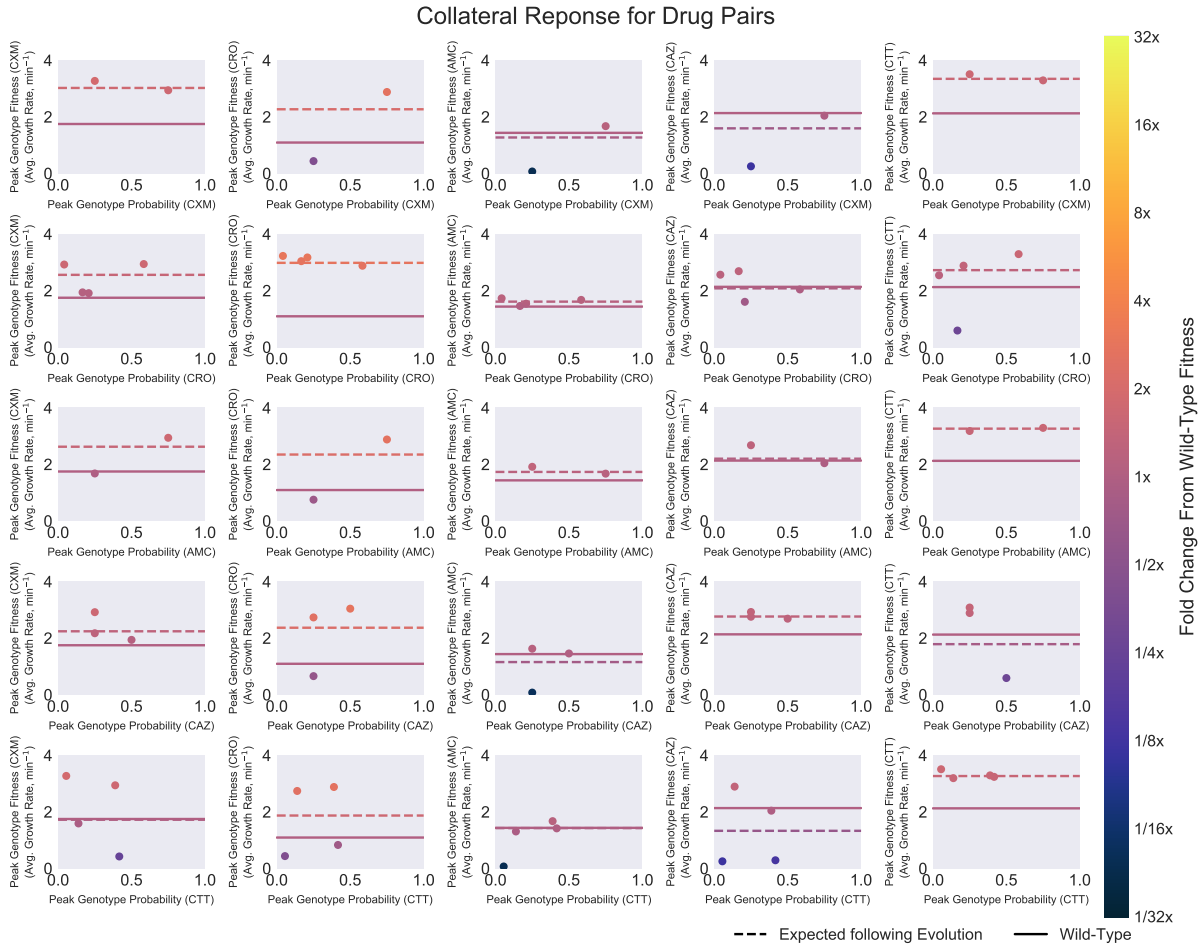

Figure 5. Collateral response between drugs in the mathematical model. Each subplot corresponds to a pair of drugs. Points represent accessible local optima genotypes in the landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution in the landscape of the labelled drug, and y value corresponding to the fitness of that genotype in the second landscape. The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

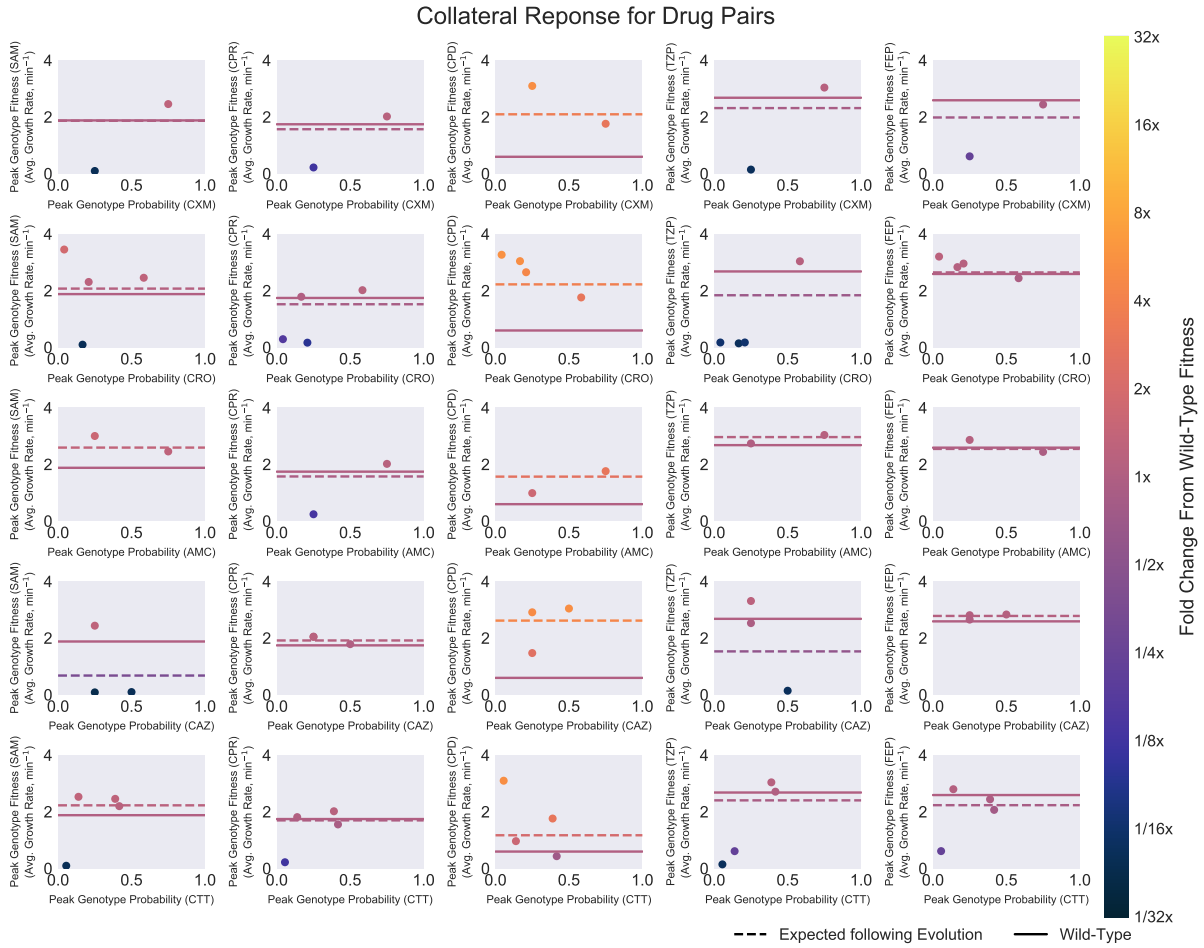

Figure 6. Collateral response between drugs in the mathematical model. Each subplot corresponds to a pair of drugs. Points represent accessible local optima genotypes in the landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution in the landscape of the labelled drug, and y value corresponding to the fitness of that genotype in the second landscape. The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

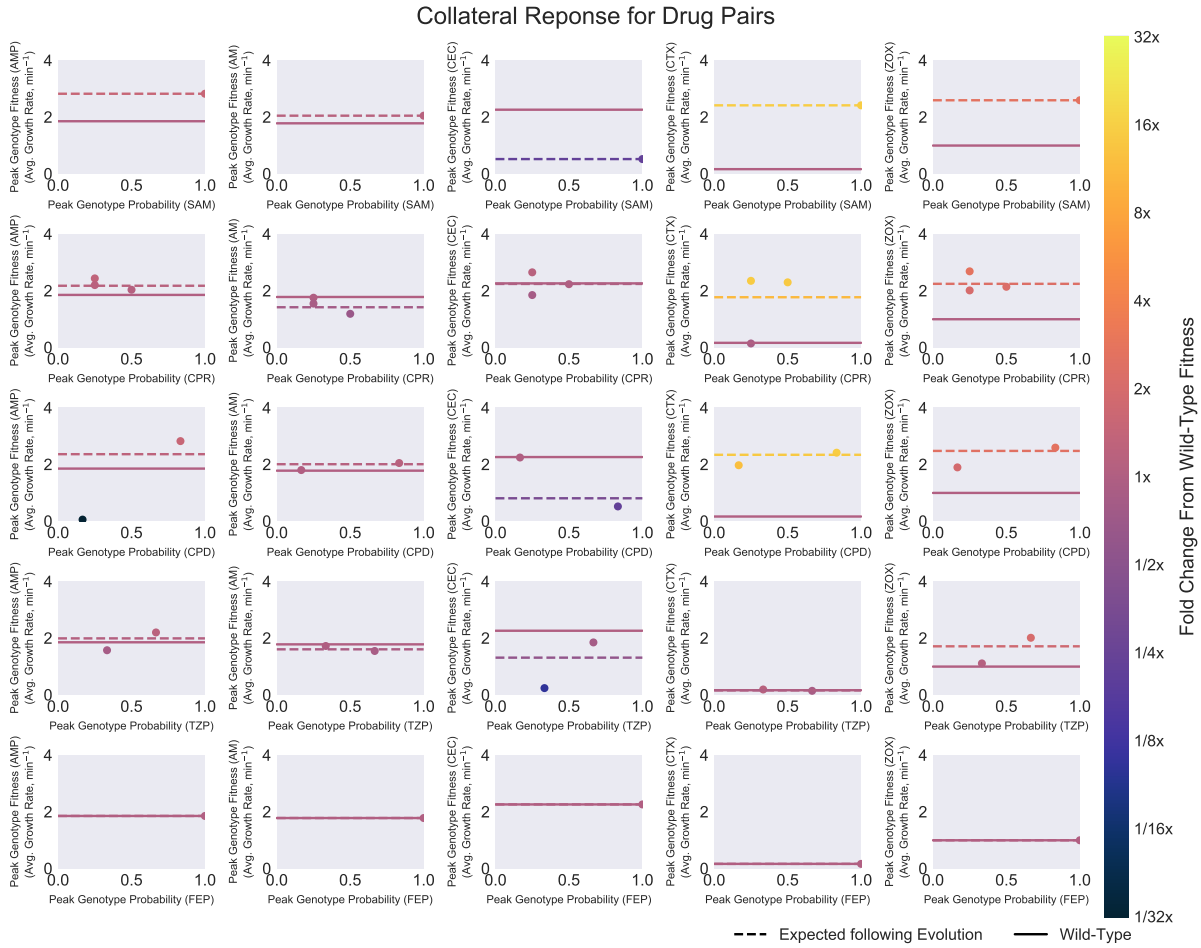

Figure 7. Collateral response between drugs in the mathematical model. Each subplot corresponds to a pair of drugs. Points represent accessible local optima genotypes in the landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution in the landscape of the labelled drug, and y value corresponding to the fitness of that genotype in the second landscape. The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

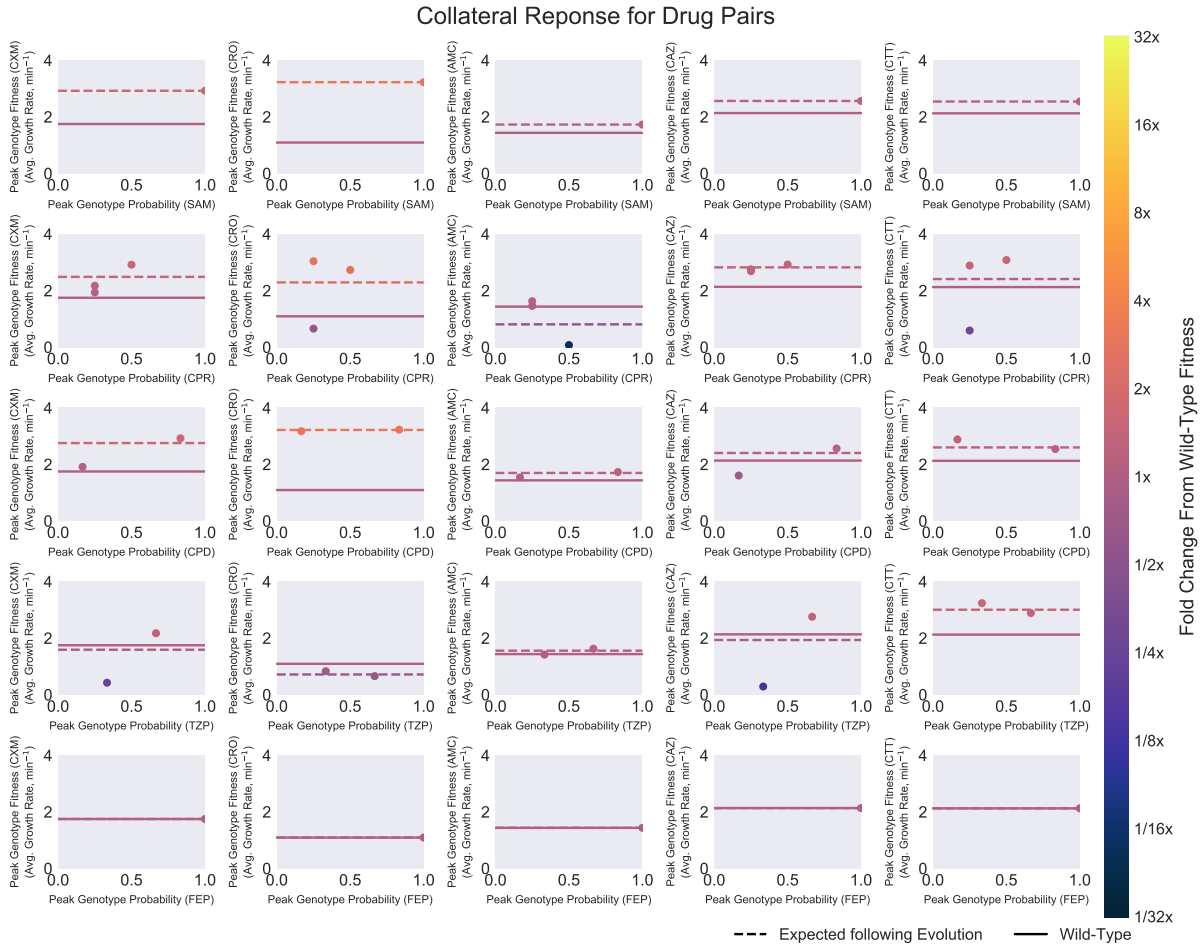

Figure 8. Collateral response between drugs in the mathematical model. Each subplot corresponds to a pair of drugs. Points represent accessible local optima genotypes in the landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution in the landscape of the labelled drug, and y value corresponding to the fitness of that genotype in the second landscape. The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

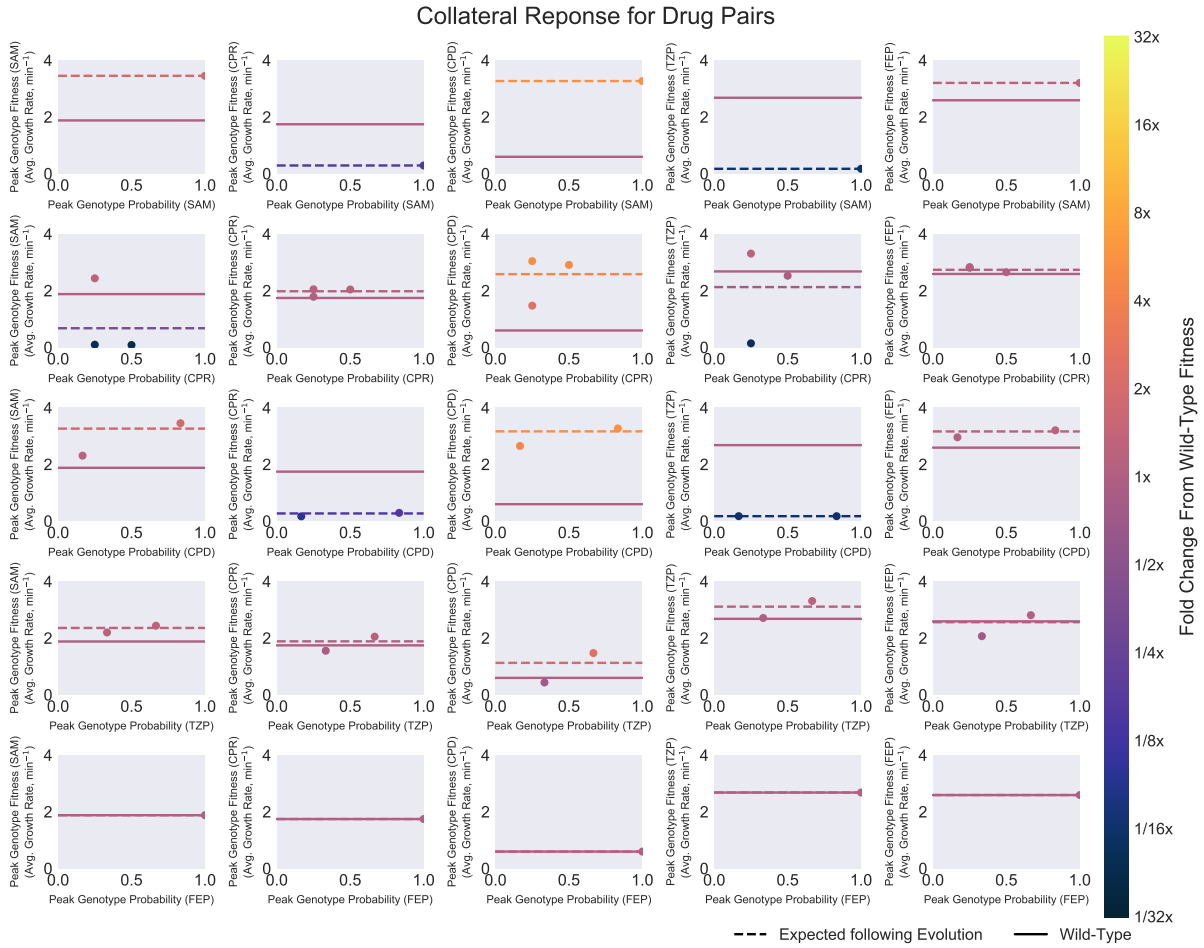

Figure 9. Collateral response between drugs in the mathematical model. Each subplot corresponds to a pair of drugs. Points represent accessible local optima genotypes in the landscape indicated on the x-axis. Each point has x value corresponding to the likelihood that it arises from evolution in the landscape of the labelled drug, and y value corresponding to the fitness of that genotype in the second landscape. The wild–type ($g = 0000$) fitness (solid line) and expected fitness following evolution under the first drug (dashed) line are shown. Lines and points are coloured according to the fold–change from wild–type fitness (colour bar).

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