

The role of aneuploidy in the evolution of cancer drug resistance

Remus Stana¹, Uri Ben-David², Daniel B. Weissman³, and Yoav Ram^{1,*}

¹School of Zoology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

²Department of Human Molecular Genetics and Biochemistry, Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

³Department of Physics, Emory University, Atlanta, GA

*Corresponding author: yoav@yoavram.com

July 23, 2023

Abstract

12 Introduction

Aneuploidy in cancer. Chromosomal instability (CIN) is the mitotic process in which cells suffer from chromosome mis-segregation that leads to aneuploidy, where cells are characterized by structural changes of the chromosomes and copy number alterations (Schukken and Foijer, 2018). Interestingly, aberrations in chromosome copy number have been shown to allow cancer cells to survive under stressful conditions such as drug therapy. Indeed, cancer cells are often likely to be aneuploid, and aneuploidy is associated with poor patient outcomes (Ben-David and Amon, 2020).

The role of chromosomal instability (CIN) in the emergence of cancer has been studied extensively in the past decades (Christine et al., 2018, Komarova et al., 2003, Michor et al., 2005, Nowak et al., 2002, Pavelka et al., 2010, Zhu et al., 2018). One hypothesis is that CIN facilitates tumor genesis by accelerating the removal of tumor suppression genes (TSG) and subsequent appearance of cancer. The deletion of tumor suppression genes can happen in two ways: two point mutations deleting both alleles of the TSG (assuming a diploid genotype), or one point mutation and one chromosomal loss event. Initial theoretical studies have shown that aneuploidy can have a significant role in the deletion of the the tumor suppressing genes when compared to two consecutive point mutations (Komarova et al., 2008, 2003, Michor et al., 2005, Nowak et al., 2002). However, when taking into account that the appearance of aneuploidy requires a mutation to trigger CIN, the probability that CIN precedes tumor genesis is highly unlikely.

Evolutionary rescue. Populations adapted to a certain environment are vulnerable to environmental changes, which might cause extinction of the population. Examples of such environmental changes include climate change, invasive species or the onset of drug therapies. Adaptation is a race against time as the population size decreases in the new environment (Tanaka and Wahl, 2022). *Evolutionary rescue* is the process where the population acquires a trait that increases fitness in the new environment such that extinction is averted. It is mathematically equivalent to the problem of crossing of fitness valley (Weissman et al., 2009, 2010). There are three potential ways for a population to survive environmental change: migration to a new habitat similar to the one before the onset of environmental change (Cobbold and Stana, 2020); adaptation by phenotypic plasticity without genetic modification (Carja and Plotkin, 2017, 2019, Levien et al., 2021); and adaptation through genetic modifications, e.g., mutation (Uecker and Hermisson, 2011, 2016, Uecker et al., 2014).

Models of evolutionary rescue usually assume that the fitness of the wildtype and mutant are homogeneous in time. An exception was given by Marrec and Bitbol (2020), who modeled the fitness of the wildtype and mutant as time dependent. Additionally, Uecker and Hermisson (2011) investigated the probability of fixation of a beneficial mutation in a variable environment with arbitrary time-dependent selection coefficient and population size. Most models focus on the probability that at least one mutation rescues the population. How multiple mutations contribute to the survival of the population is less explored, but Wilson et al. (2017) have shown that evolutionary rescue is significantly enhanced by soft selective sweeps when multiple mutations contribute. Evolutionary rescue that requires two successive mutations has been investigated using diffusion approximation by Martin et al. (2013).

Methods

52 Evolutionary model

We follow the number of cancer cells that have one of three different genotypes at time t : wildtype, w_t ; aneuploid, a_t ; and mutant, m_t . These cells divide and die with rates λ_k and μ_k (for $k = w, a, m$). The difference between the division and death rate is $\Delta_k = \lambda_k - \mu_k$. We assume the population of cells is under a strong stress, such as drug therapy, to which the wildtype genotype is susceptible and

therefore $\Delta_w < 0$, whereas the mutant is resistant to the stress, $\Delta_m > 0$. We analyze three scenarios:
 58 in the first, aneuploid cells are partially resistant, $\Delta_m > \Delta_a > 0$; in the second, aneuploid cells are
 tolerant, $0 > \Delta_a > \Delta_w$ (see Brauner et al., 2016, for the distinction between susceptible, resistant,
 60 and tolerant); in the third, aneuploid cells are non-growing or "barely growing", that is, either slightly
 tolerant or slightly resistant, such that $\Delta_a \approx 0$. Wildtype cells may missegregate to become aneuploids
 62 at rate u . Both aneuploid and wildtype cells may mutate to become mutants at rate v , which we assume
 is lower than the division rates, $v < \min(\lambda_w, \lambda_a, \lambda_m)$. See Figure 1 for an illustration of the model.

64 Stochastic simulations

Simulations are performed using a *Gillespie algorithm* (Gillespie, 1976, 1977) implemented in Python
 66 (Van Rossum and Others, 2007). The simulation monitors the number of cells of each type: wildtype,
 aneuploid, and mutant. The wildtype population initially consists of w_0 cells, whereas the other cell
 68 types are initially absent.

The state of the stochastic system at time t is represented by the triplet (w_t, a_t, m_t) . The following
 describes the events that may occur (right column), the rates at which they occur (middle column),
 and the effect these events have on the state (Figure 1):

$(+1, 0, 0)$	$\lambda_w w_t$	(birth of wildtype cell),
$(-1, 0, 0)$	$\mu_w w_t$	(death of wildtype cell),
$(-1, +1, 0)$	$u w_t$	(wildtype cell becomes aneuploid),
$(-1, 0, +1)$	$v w_t$	(wildtype cell becomes mutant),
$(0, +1, 0)$	$\lambda_a a_t$	(birth of aneuploid cell),
$(0, -1, 0)$	$\mu_a a_t$	(death of aneuploid cell),
$(0, -1, +1)$	$v a_t$	(aneuploid cell becomes mutant),
$(0, 0, +1)$	$\lambda_m m_t$	(birth of mutant cell),
$(0, 0, -1)$	$\mu_m m_t$	(death of mutant cell).

Each iteration of the simulation loop starts with computing the rates ν_j of each event j . We then
 draw the time until the next event, Δt , from an exponential distribution whose rate parameter is the
 sum of the rates of all events, such that $\Delta t \sim \text{Exp}(\sum_j \nu_j)$. Then, we randomly determine which event
 occurred, where the probability for event j is $p_j = \nu_j / \sum_i \nu_i$. Finally, we update the number of cells of
 each type according to the event that occurred and update the time from t to $t + \Delta t$. We repeat these
 iterations until either the population becomes extinct (the number of cells of all types is zero) or the
 number of mutant cells is high enough so that its extinction probability is $< 0.1\%$, that is until

$$m_t > \left\lceil \frac{3 \log 10}{\log(\lambda_m / \mu_m)} \right\rceil + 1.$$

τ -leaping. When simulations are slow (e.g. due to large population size), we utilize τ -leaping
 70 (Gillespie, 2001), where change in number of cells of genotype i in a fixed time interval Δt is
 Poisson distributed with mean $\nu_i \Delta t$. If the change in number of cells is negative and larger than the
 72 subpopulation size then the subpopulation size is updated to be zero.

Density-dependent growth. In our analysis we assume that lineages produced by cells from the
 initial population divide and die independently of each other, which may be unrealistic, as cells
 usually compete for resources. A more realistic model includes competition for limited resources and
 spatial structure, which may play an important role in the development of cancer (e.g., Martens et al.,

2011). To simulate birth and death rates that depend on the number of cells in the population, we transform the rates of division and death to the following:

$$\begin{aligned}\lambda'_w &= \lambda_w, \\ \mu'_w &= \mu_w, \\ \lambda'_a &= C_1 + (\lambda_a - \mu_a) \left(1 - \frac{w + a + m}{K}\right), \\ \mu'_a &= C_1, \\ \lambda'_m &= C_2 + (\lambda_m - \mu_m) \left(1 - \frac{w + a + m}{K}\right), \\ \mu'_m &= C_2,\end{aligned}$$

where $C_1, C_2 > 0$ are constants and K is the maximum carrying capacity.

74 Code and data availability.

All source code is available online at <https://github.com/yoavram-lab/EvolutionaryRescue>.

76 Results

Evolutionary rescue probability

78 In our model, *evolutionary rescue* occurs when resistant cells appear and fixate ($m_t \gg 1$) in the
 80 population before the population becomes extinct ($w_t = a_t = m_t = 0$). Aneuploidy may contribute
 to evolutionary rescue by either preventing (when $\Delta_a > 0$) or delaying (when $0 > \Delta_a > \Delta_w$) the
 82 extinction of the population before mutant cells appear and fixate. We assume independence between
 clonal lineages starting from an initial population of N wildtype cells (we check the effect of density-
 dependent growth on our results below). We therefore define p_w as the probability that a lineage
 84 starting from a single wildtype cell avoids extinction by acquiring drug resistance. Thus, $N^* = 1/p_w$
 is the threshold tumor size above which evolutionary rescue is very likely, and the rescue probability
 86 is given by

$$p_{\text{rescue}} = 1 - (1 - p_w)^N \approx 1 - e^{-Np_w} = 1 - e^{-N/N^*}. \quad (2)$$

88 where the approximation $(1 - p_w) \approx e^{-p_w}$ assumes that p_w (but not necessarily Np_w) is small. Indeed,
 when $N < 1/p_w$, then the probability for evolutionary rescue is $p_{\text{rescue}} \approx Np_w$ and when $N > 1/p_w$,
 90 it is $p_{\text{rescue}} \approx 1$, justifying the definition of N^* as the threshold tumor size.

92 In the Appendix, we use the theory of multi-type branching processes to find approximate expres-
 sions eqs. (12), (19) and (22) for p_w in different regimes. Substituting these into $N^* = 1/p_w$, we find
 94 approximations for the threshold tumor size, N^* . For these approximations, an important quantity is
 $T^* = (4\nu\lambda_a\Delta_m/\lambda_m)^{-1/2}$, which is the critical time an aneuploid lineage needs to survive to produce
 96 a resistant mutant that avoids random extinction. First, if aneuploidy is very rare ($uT^* < 1$), or if
 aneuploidy is rare ($u < -\Delta_a$) and very sensitive to the drug ($\Delta_a T^* < -1$), then rescue will likely occur
 98 by a direct resistance mutation in a sensitive cell, such that

$$N_m^* \approx \frac{|\Delta_w|}{\nu} \cdot \frac{\lambda_m}{\Delta_m}. \quad (3)$$

100 Here, $|\Delta_w|/\nu$ is the ratio of the rate at which wild-type cells are decreasing in number and the rate at
 which they are mutating.

102 Otherwise, aneuploidy is frequent enough ($u > \max(-\Delta_a, 1/T^*)$) to affect the evolution of drug
 resistance. The threshold tumor size, N^* , can then be approximated by one of the following cases,
 104 depending on $\Delta_a T^*$, the change in the log of the aneuploid population size during the critical time,

$$N_a^* \approx \frac{|\Delta_w|}{u} \cdot \begin{cases} \frac{|\Delta_a|}{v} \cdot \frac{\lambda_m}{\Delta_m}, & \Delta_a T^* \ll -1, \\ 2\lambda_a T^*, & -1 \ll \Delta_a T^* \ll 1, \\ \frac{\lambda_a}{\Delta_a}, & \Delta_a T^* \gg 1. \end{cases} \quad (4)$$

106 Comparing these approximations to results of stochastic evolutionary simulations, we find that the
 approximations perform very well (Figures 2 to 4).

108 Using eqs. (3) and (4), we can find the ratio of threshold tumor size for rescue via aneuploidy (u
 is high) or via direct mutation (u is low),

$$\frac{N_a^*}{N_m^*} \approx \begin{cases} \frac{|\Delta_a|}{u}, & \Delta_a T^* \ll -1, \\ \frac{1}{u} \left(\frac{\lambda_a}{v} \cdot \frac{\lambda_m}{\Delta_m} \right)^{1/2}, & -1 \ll \Delta_a T^* \ll 1, \\ v \frac{\Delta_m}{\lambda_m} \cdot \left(u \frac{\Delta_a}{\lambda_a} \right)^{-1}, & \Delta_a T^* \gg 1. \end{cases} \quad (5)$$

In all cases, the effect of aneuploidy increases (i.e., the threshold size ratio decreases) when the
 112 aneuploidy rate u increases. Increasing the aneuploid growth rate Δ_a also leads to an increased role
 of aneuploidy, but only in the first and third case.

114 In the first case, $|\Delta_a|/u$ is the ratio of the expected time for an aneuploid lineage to appear, $1/u$, and
 the expected time until that lineage disappears, $1/\Delta_a$. In the third case, $\left(v \frac{\Delta_m}{\lambda_m}\right) / \left(u \frac{\Delta_a}{\lambda_a}\right)$ is the ratio of
 116 the rates of formation of resistant mutants that avoid extinction and partially resistant aneuploids that
 avoid extinction. In the second case, $\frac{1}{u} \left(\frac{\lambda_a}{v} \cdot \frac{\lambda_m}{\Delta_m} \right)^{1/2} = \sqrt{\frac{\Delta_a}{u} \cdot v \frac{\Delta_m}{\lambda_m} \cdot \left(u \frac{\Delta_a}{\lambda_a}\right)^{-1}}$, which is the geometric
 118 mean of the first and third cases.

Interestingly, increasing both the aneuploid division rate, λ_a , and the aneuploid death rate, μ_a ,
 120 such that the growth rate Δ_a remains constant, leads to decreases in T^* , and therefore to the second
 case. In this case, increasing the division rate λ_a should also increase the mutation rate v in aneuploid
 122 cells, as mutations mostly occur during division, so overall the threshold tumor size N_a^* is unaffected
 by the division rate λ_a (i.e., $d\lambda_a T^*/d\lambda_a = 0$). Thus, if aneuploids rapidly die due to the drug but
 124 compensate by rapidly dividing, further increasing the division rate will *not* facilitate adaptation.

Density-dependent growth. In our analysis we used branching processes, which assume that growth
 126 (division and death) is density-independent. However, growth may be limited by resources (oxygen,
 nutrients, etc.) and therefore depend on cell density. We therefore performed stochastic simulations
 128 of a logistic growth model with carrying capacity K (see Methods). We find that our approximations
 agree with results of simulations with density-dependent growth for biologically relevant parameter
 130 values (Figure 4).

Standing vs. de-novo genetic variation. In the above we assumed that at the onset of drug treatment,
 132 the initial tumor consisted entirely of wildtype cells that are drug sensitive. However, aneuploid cells
 are likely generated even before onset of treatment at some rate $\tilde{u} \leq u$ (because the treatment itself
 134 may promote generation of aneuploid cells REF), which are likely to have a deleterious effect in the
 absence of the drug, s (REF). But if the number of cells in the tumor N is large (as expected if the
 136 tumor is to be treated with a drug), there may already be a fraction $f \approx \tilde{u}/s$ of aneuploid cells in the
 population.

138 Therefore, the threshold tumor size with standing generation variation, \tilde{N}_a^* , is similar to the ratio
 with de-novo variation, N_a^* , except that the sensitive growth rate $|\Delta_w|$ is replaced with the aneuploidy
 140 cost, s , such that

$$\frac{\tilde{N}_a^*}{N_a^*} = \frac{u}{\tilde{u}} \frac{s}{|\Delta_w|}. \quad (6)$$

142 Therefore, standing genetic variation will drive adaptation to the drug if Δ_w is very negative due
 to a stronger effect of the drug on sensitive cells, or if s is very small due to a low cost of aneuploidy
 144 in the pre-drug conditions. In contrast, de-novo aneuploids will have a stronger effect on adaptation if
 the aneuploidy cost s is large, the effect of the drug is weak (Δ_w is small), or if the drug induces the
 146 appearance of aneuploid cells ($u > \tilde{u}$).

Recurrence time due to evolutionary rescue

148 Even when evolutionary rescue occurs and leads to recurrence of the tumor, it may take a long time.
 The overall expected recurrence time can be estimated by adding two terms: the mean waiting time
 150 for evolutionary rescue—the appearance of a resistant lineage that avoid extinction—and the expected
 time for proliferation of that lineage back to the original tumor size, N .

Evolutionary rescue time. In Appendix C we derive an approximation for τ_1 , the expected rescue
 time without aneuploidy ($u = 0$), and τ_2 , the expected rescue time with aneuploidy ($u > 0$),

$$\tau_1 \approx \frac{1 + e^{-Np_w}}{uNp_a}, \quad (7)$$

$$\tau_2 \approx \frac{1 + e^{-Np_w}}{uNp_m}, \quad (8)$$

152 Figure 7 shows the agreement between this approximations and simulation results for intermediate
 and large tumor sizes.

Thus, the ratio of the expected rescue time with and without aneuploidy ($u > 0$ and $u = 0$,
 respectively),

$$\frac{\tau_1}{\tau_2} \approx \frac{p_a}{p_m} = \frac{1}{2} \frac{\lambda_m}{\Delta_m} \left[\left(\frac{\lambda_a}{\Delta_a} \right)^{-1} + \sqrt{\left(\frac{\lambda_a}{\Delta_a} \right)^{-2} + 4v \left(\lambda_a \frac{\lambda_m}{\Delta_m} \right)^{-1}} \right]. \quad (9)$$

154 Figure 8 shows how this ratio, τ_1/τ_2 , changes as a function of the initial tumor size, N , for different
 aneuploid growth rates, Δ_a .

156 Importantly, in Figure 7 we observe that in initially large tumors, direct mutation drives evolutionary
 rescue while aneuploidy plays a role for intermediate sized tumors. This is consistent with Figure 6B,D,
 158 where aneuploidy only increases the probability of evolutionary rescue in small and intermediate
 tumors ($N < 10^7$).

160 **Proliferation time.** TODO

Discussion

162 We have modeled a tumor—a population of cancer cells—exposed to drug treatment that causes the pop-
 ulation to decline in size towards potential extinction. In this scenario, the tumor can be "evolutionary
 164 rescued", or escape extinction, via two paths. In the direct path, a sensitive cell acquires a mutation
 that confers resistance that allows it to rapidly grow. In the indirect path, a sensitive cell first becomes

166 aneuploid, which diminishes the effect of the drug, and then an aneuploid cell acquires a mutation that confers resistance (Figure 1).

168 Using multitype branching processes, we derived the probability of evolutionary rescue of the
population of cancer cells under different scenarios for the effect of aneuploidy, ranging from tolerance
170 to partial resistance. We obtained exact and approximate expressions for the probability of evolutionary
rescue (eq. (2)). Our results show that the probability of evolutionary rescue increases with the initial
172 tumor size N , the sensitive growth rate Δ_w , the mutation rate ν , and the aneuploidy rate u .

When aneuploid cells are partially resistant to the drug ($\Delta_w \ll 0 \ll \Delta_a \ll \Delta_m$), evolutionary
174 rescue can be approximated by a one-step process in which aneuploidy itself rescues the population
(Figure 2). When aneuploidy only provides tolerance to the drug ($\Delta_w \ll \Delta_a \ll 0 \ll \Delta_m$), it cannot
176 rescue the population. Instead, it acts as a *stepping stone* through which the resistant mutant can
appear more rapidly, given that the aneuploid cell population declines slower than the sensitive cell
178 population. In this case, aneuploidy provides two benefits. First, it delays the extinction of the
population—providing more time for appearance of the resistance mutation. Second, it increases the
180 population size relative to a sensitive population—providing more cells in which mutations can occur,
i.e., it increases the mutation supply, $N\nu$.

182 We find that aneuploidy can have a significant effect on evolutionary rescue (Figures 6 and 8). For
example, when aneuploidy cells are "barely-resistant" (they grow at a very low rate, $\Delta_a = 10^{-3}$) the
184 probability of evolutionary rescue is 1,000-fold higher with aneuploidy than without it (for parameters
previously described in cancer, see Table 1). Interestingly, aneuploidy is unlikely to contribute to
186 evolutionary rescue in primary tumors in which the number of cells is large enough ($N > 10^7$) for the
appearance of resistant mutation directly in sensitive cells before these cells become extinct (Figure 6).
188 However, aneuploidy can have a crucial role in evolutionary rescue of secondary tumors, in which the
number of sensitive cells may be below the detection threshold of $\sim 10^7$ (Bozic et al., 2013). Given
190 the fact that the mean time for such secondary tumors to overcome chemotherapy can be of the order
of 100 days (Figure 7), this can explain the reappearance of cancer even after initial remission. Indeed,
192 we find that the tumor size can decrease by orders of magnitude before it is rescued (Figure 9).

We hypothesized that presence of *standing variation*—the existence of a subpopulation of aneuploid
194 cancer cells before therapy begins—can facilitate evolutionary rescue by reducing the waiting time for
the appearance of aneuploid cells. Indeed, we observe that even when a small fraction of the initial
196 tumor is aneuploid, evolutionary rescue is more likely to occur through this existing standing variation,
rather than through *de novo* aneuploid cells (Figure 5).

198 We have assumed that cancer cell lineages are independent of each other. However, this may not
be the case, as cancer cells compete for resources (e.g., blood supply). Nevertheless, we find that when
200 the carrying capacity is large our approximation for the probability of evolutionary rescue agrees with
results of stochastic simulations with density-dependent growth (Figure 4). Future work may focus on
202 scenarios with small carrying capacity by analyzing density-dependent branching processes.

Our model predictions may be tested by experiments (Martin et al., 2013). For example, to study
204 the effects of initial tumor size on the probability of evolutionary rescue, a large culture mass can be
propagated from a single cancer cell in permissive conditions and then diluted to a range of starting
206 tumor sizes. Afterwards, these tumors may be exposed to anti-cancer drugs that induces aneuploidy
or to saline solution for control. Cell density can then be measured and compared to the predictions
208 of our model.

Acknowledgements

210 This work was supported in part by the Israel Science Foundation (ISF 552/19, YR), the US–Israel Binational
Science Foundation (BSF 2021276, YR), Minerva Stiftung Center for Lab Evolution (YR), and the Ela Kodesz
212 Institute for Research on Cancer Development and Prevention (RS).

References

- Bakker, B., Schubert, M., Bolhaqueiro, A. C., Kops, G. J., Spierings, D. C. and Foijer, F. (2023), ‘Predicting cin rates from single-cell whole genome sequencing data using an in silico model’, *bioRxiv* pp. 2023–02.
- Ben-David, U. and Amon, A. (2020), ‘Context is everything: aneuploidy in cancer’, *Nature Reviews Genetics* **21**(1), 44–62.
- Bozic, I., Reiter, J. G., Allen, B., Antal, T., Chatterjee, K., Shah, P., Moon, Y. S., Yaquibie, A., Kelly, N., Le, D. T. et al. (2013), ‘Evolutionary dynamics of cancer in response to targeted combination therapy’, *elife* **2**, e00747.
- Brauner, A., Fridman, O., Gefen, O. and Balaban, N. Q. (2016), ‘Distinguishing between resistance, tolerance and persistence to antibiotic treatment’, *Nature Reviews Microbiology* **14**(5), 320–330.
- Carja, O. and Plotkin, J. B. (2017), ‘The evolutionary advantage of heritable phenotypic heterogeneity’, *Scientific reports* **7**(1), 1–12.
- Carja, O. and Plotkin, J. B. (2019), ‘Evolutionary rescue through partly heritable phenotypic variability’, *Genetics* **211**(3), 977–988.
- Christine, J. Y., Regan, S., Liu, G., Alemara, S. and Heng, H. H. (2018), ‘Understanding aneuploidy in cancer through the lens of system inheritance, fuzzy inheritance and emergence of new genome systems’, *Molecular cytogenetics* **11**(1), 1–13.
- Cobbold, C. A. and Stana, R. (2020), ‘Should I stay or should I go: partially sedentary populations can outperform fully dispersing populations in response to climate-induced range shifts’, *Bulletin of Mathematical Biology* **82**(2), 1–21.
- Del Monte, U. (2009), ‘Does the cell number 10⁹ still really fit one gram of tumor tissue?’, *Cell cycle* **8**(3), 505–506.
- Gillespie, D. T. (1976), ‘A general method for numerically simulating the stochastic time evolution of coupled chemical reactions’, *Journal of computational physics* **22**(4), 403–434.
- Gillespie, D. T. (1977), ‘Exact stochastic simulation of coupled chemical reactions’, *The journal of physical chemistry* **81**(25), 2340–2361.
- Gillespie, D. T. (2001), ‘Approximate accelerated stochastic simulation of chemically reacting systems’, *The Journal of chemical physics* **115**(4), 1716–1733.
- Harris, T. E. et al. (1963), *The theory of branching processes*, Vol. 6, Springer Berlin.
- Komarova, N. L., Sadovsky, A. V. and Wan, F. Y. (2008), ‘Selective pressures for and against genetic instability in cancer: a time-dependent problem’, *Journal of The Royal Society Interface* **5**(18), 105–121.
- Komarova, N. L., Sengupta, A. and Nowak, M. A. (2003), ‘Mutation–selection networks of cancer initiation: tumor suppressor genes and chromosomal instability’, *Journal of theoretical biology* **223**(4), 433–450.
- Levien, E., Min, J., Kondev, J. and Amir, A. (2021), ‘Non-genetic variability in microbial populations: survival strategy or nuisance?’, *Reports on Progress in Physics* **84**(11), 116601.

- Marrec, L. and Bitbol, A.-F. (2020), ‘Adapt or perish: Evolutionary rescue in a gradually deteriorating environment’, *Genetics* **216**(2), 573–583.
- Martens, E. A., Kostadinov, R., Maley, C. C. and Hallatschek, O. (2011), ‘Spatial structure increases the waiting time for cancer’, *New journal of physics* **13**(11), 115014.
- Martin, G., Aguilée, R., Ramsayer, J., Kaltz, O. and Ronce, O. (2013), ‘The probability of evolutionary rescue: towards a quantitative comparison between theory and evolution experiments’, *Philosophical Transactions of the Royal Society B: Biological Sciences* **368**(1610), 20120088.
- Michor, F., Iwasa, Y., Vogelstein, B., Lengauer, C. and Nowak, M. A. (2005), Can chromosomal instability initiate tumorigenesis?, in ‘Seminars in cancer biology’, Vol. 15, Elsevier, pp. 43–49.
- Nowak, M. A., Komarova, N. L., Sengupta, A., Jallepalli, P. V., Shih, I.-M., Vogelstein, B. and Lengauer, C. (2002), ‘The role of chromosomal instability in tumor initiation’, *Proceedings of the National Academy of Sciences* **99**(25), 16226–16231.
- Nowak, M. A., Michor, F., Komarova, N. L. and Iwasa, Y. (2004), ‘Evolutionary dynamics of tumor suppressor gene inactivation’, *Proceedings of the National Academy of Sciences* **101**(29), 10635–10638.
- Pavelka, N., Rancati, G. and Li, R. (2010), ‘Dr Jekyll and Mr Hyde: role of aneuploidy in cellular adaptation and cancer’, *Current opinion in cell biology* **22**(6), 809–815.
- Schukken, K. M. and Foijer, F. (2018), ‘Cin and aneuploidy: different concepts, different consequences’, *Bioessays* **40**(1), 1700147.
- Tanaka, M. M. and Wahl, L. M. (2022), ‘Surviving environmental change: when increasing population size can increase extinction risk’, *Proceedings of the Royal Society B* **289**(1976), 20220439.
- Uecker, H. and Hermisson, J. (2011), ‘On the fixation process of a beneficial mutation in a variable environment’, *Genetics* **188**(4), 915–930.
- Uecker, H. and Hermisson, J. (2016), ‘The role of recombination in evolutionary rescue’, *Genetics* **202**(2), 721–732.
- Uecker, H., Otto, S. P. and Hermisson, J. (2014), ‘Evolutionary rescue in structured populations’, *The American Naturalist* **183**(1), E17–E35.
- Uecker, H., Setter, D. and Hermisson, J. (2015), ‘Adaptive gene introgression after secondary contact’, *Journal of mathematical biology* **70**, 1523–1580.
- Van Rossum, G. and Others (2007), Python Programming Language., in ‘USENIX Annu. Tech. Conf.’.
- Weissman, D. B., Desai, M. M., Fisher, D. S. and Feldman, M. W. (2009), ‘The rate at which asexual populations cross fitness valleys’, *Theoretical population biology* **75**(4), 286–300.
- Weissman, D. B., Feldman, M. W. and Fisher, D. S. (2010), ‘The rate of fitness-valley crossing in sexual populations’, *Genetics* **186**(4), 1389–1410.
- Wilson, B. A., Pennings, P. S. and Petrov, D. A. (2017), ‘Soft selective sweeps in evolutionary rescue’, *Genetics* **205**(4), 1573–1586.
- Zhu, J., Tsai, H.-J., Gordon, M. R. and Li, R. (2018), ‘Cellular stress associated with aneuploidy’, *Developmental cell* **44**(4), 420–431.

	Name	Value	Units	References
N	Initial tumor size	$10^7 - 10^9$	cells	Del Monte (2009)
λ_w	Wildtype division rate	0.14	1/days	Bozic et al. (2013)
μ_w	Wildtype death rate	0.17	1/days	Bozic et al. (2013)
λ_a	Aneuploid division rate*	0.14	1/days	-
μ_a	Aneuploid death rate*	0.13 – 0.17	1/days	-
λ_m	Mutant division rate	0.14	1/days	Bozic et al. (2013)
μ_m	Mutant death rate	0.13	1/days	Bozic et al. (2013)
u	Missegregation rate	$10^{-3} - 10^{-2}$	1/cell division	Bakker et al. (2023), Nowak et al. (2004)
v	Mutation rate	$10^{-7} - 10^{-9}$	1/gene/cell division	Nowak et al. (2004)

Table 1: Model parameters. Aneuploid birth rate λ_a is set to the same value as the wildtype and mutant birth rates, λ_w and λ_m . Aneuploid death rate μ_a is set to an intermediate value between the wildtype and mutant death rates, μ_w and μ_m .

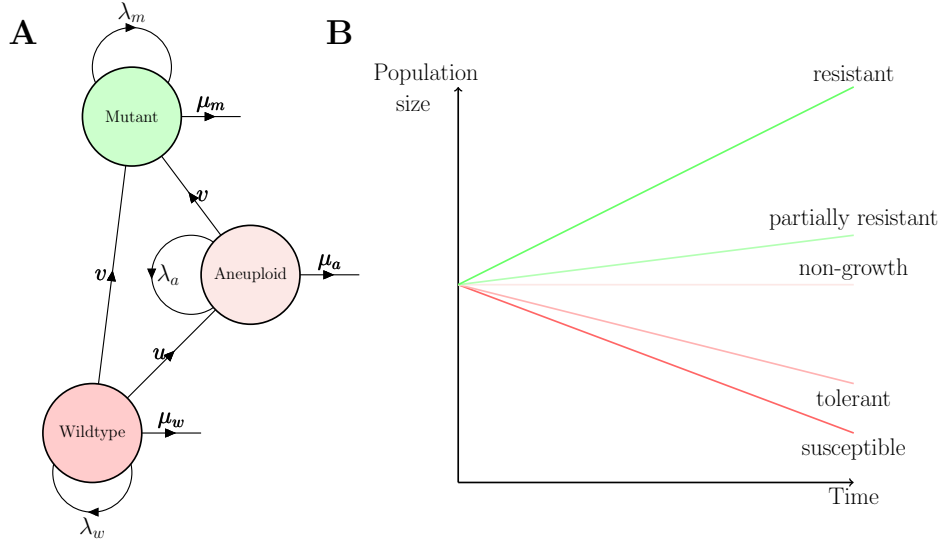


Figure 1: Model illustration. (A) A population of cancer cells is composed of wildtype, aneuploid, and mutant cells, which divide with rates λ_w , λ_a , and λ_m and die at rates μ_w , μ_a , and μ_m , respectively. Wildtype cells can become aneuploid at rate u . Both aneuploid and wildtype cells can acquire a beneficial mutation with rate v . Color denotes the relative growth rates of the three genotypes such that $\lambda_w - \mu_w < \lambda_a - \mu_a < \lambda_m - \mu_m$. (B) The wildtype and the mutant are susceptible and resistant, respectively, to the drug. The aneuploid may be tolerant, non-growing, or partially resistant.

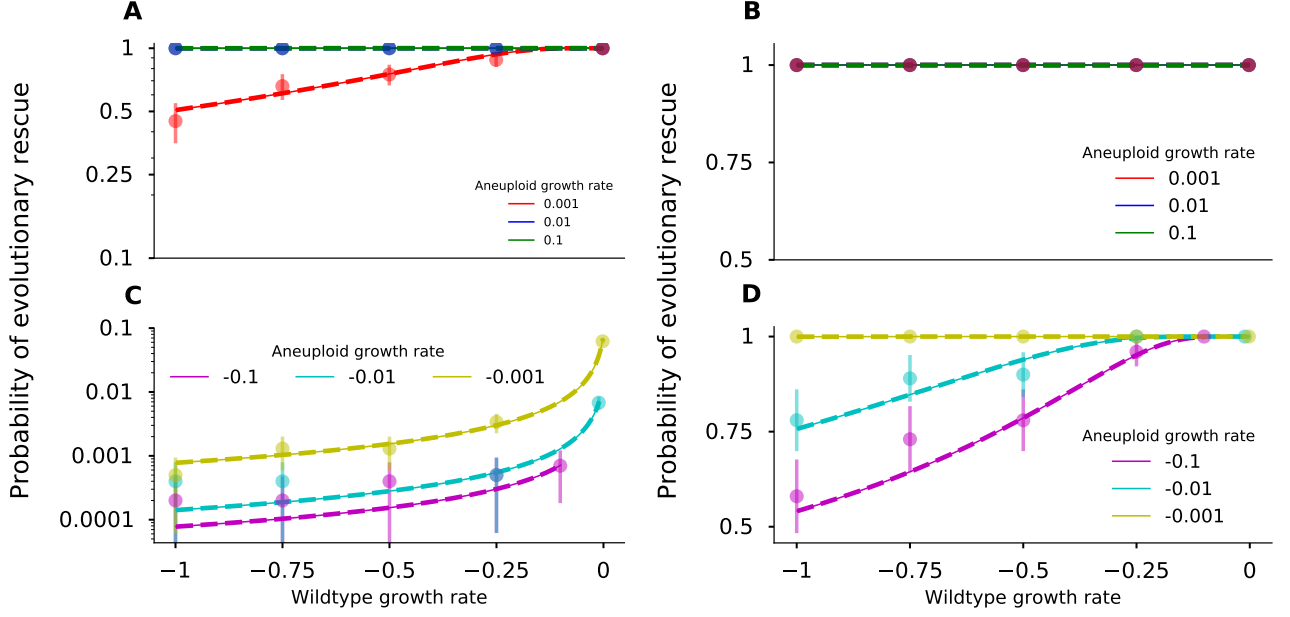


Figure 2: Evolutionary rescue probability with partially resistant or tolerant aneuploid cells. Rescue probability is very high when aneuploidy provides partial resistance ($\lambda_a = 0.01$), in an initially small tumor (**A**, $N = 10^4$) and even more so in an initially large tumor (**B**, $N = 10^8$). When aneuploidy provides tolerance (**C**, $N = 10^4$; **D**, $N = 10^8$), the rescue probability is much lower. In both scenarios, rescue probability increase with both the wildtype growth rate (x-axis) and the aneuploidy growth rate (colors). Markers represent simulation results with 95% CI; solid and dashed lines for the exact formula (eq. (11) in eq. (2)); dashed lines for the approximate formula (eq. (23)), demonstrating that they all agree. Parameters: division rate $\lambda_w = \lambda_a = \lambda_m = 0.14$ (so that growth rate changes due to variable death rate); mutant death rate $\mu_m = 0.13$ (so that mutant growth rate $\Delta_m = 0.01$); aneuploidy rate $u = 10^{-2}$; mutation rate $v = 10^{-7}$.

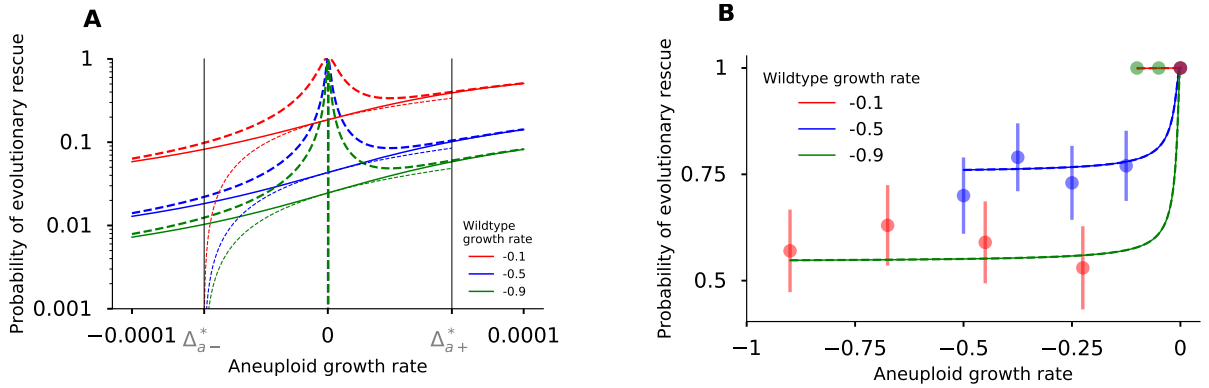


Figure 3: Evolutionary rescue probability with tolerant or non-growing aneuploid cells. Rescue probability grows with the aneuploid growth rate Δ_a (x-axis), and is much higher in an initially large tumor than in a small one (**A**: $N = 10^4$; **B**: $N = 10^8$). Markers for simulation results with 95% CI; solid lines for the exact formula (eq. (11) in eq. (2)); dashed lines for the approximate formula (eq. (23)). The approximation agrees with the simulation and exact solution when the initial tumor size is large (panel B). When the tumor size is small (panel A), we switch between the approximation for tolerant and for non-growing aneuploid cells; the switch occurs at $1/T^*$. Parameters: $\lambda_w = \lambda_a = \lambda_m = 0.14$; $\mu_m = 0.13$; $u = 10^{-2}$; $v = 10^{-7}$.

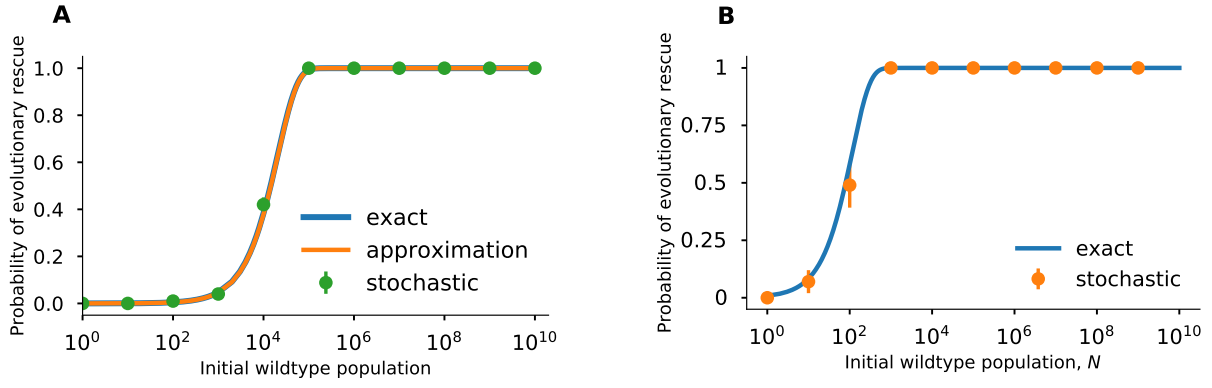


Figure 4: Evolutionary rescue probability for variable initial tumor size. (A) Comparison of simulation results (markers with 95% CI, too small to appear with 10^5 simulations per marker), the exact formula (blue line, eq. (11) in eq. (2)) and the approximate formula (orange line, eq. (23)). (B) Comparison of results of simulations with density-dependent growth (markers with 95% CI) and the exact formula (blue line, eq. (11) in eq. (2)) with maximum carrying capacity $K = 10^9$. Parameters: $\lambda_w = \lambda_a = \lambda_m = 0.14$; $\mu_w = 0.17$; (A) $\mu_a = 0.15$, (B) $\mu_a = 0.135$; $\mu_m = 0.13$; $u = 10^{-2}$; $v = 10^{-7}$.

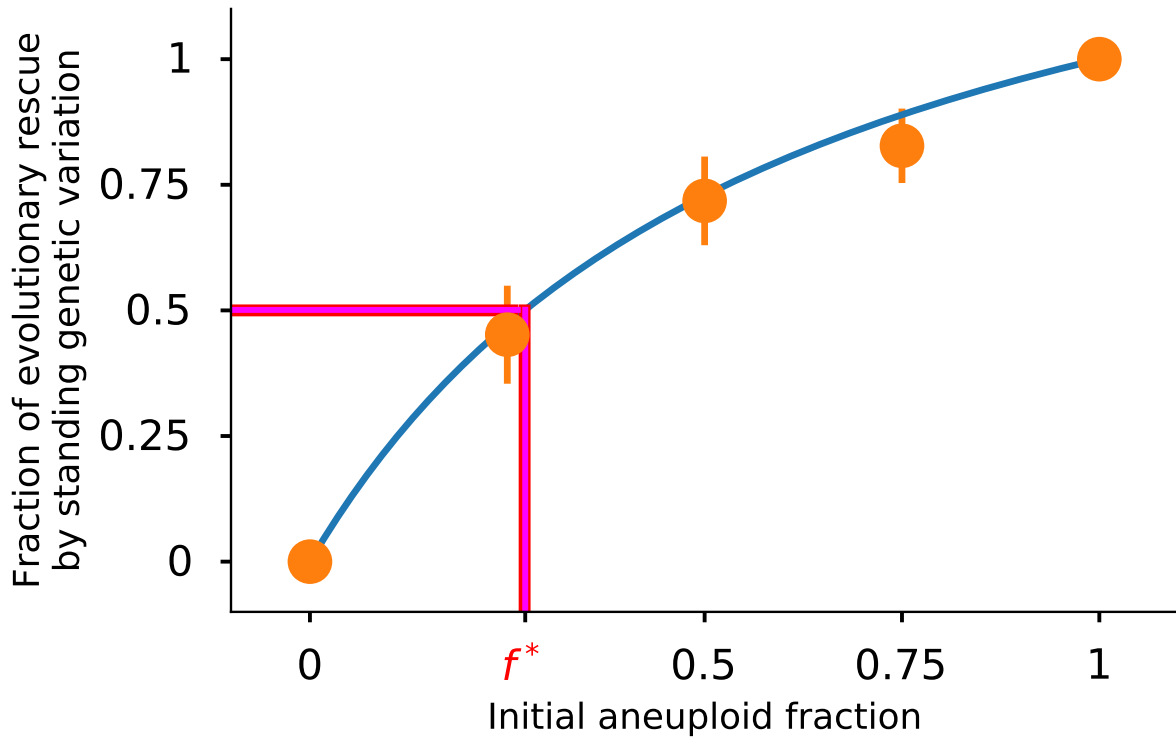


Figure 5: Effect of standing variation on evolutionary rescue. In aneuploid cells already exist in the population at the onset of drug therapy as standing genetic variation, then evolutionary rescue is more likely... Parameters: $\lambda_w = \lambda_a = \lambda_m = 0.14$; $\mu_w = 0.17$; $\mu_a = 0.145$; $\mu_m = 0.13$; $u = 10^{-2}$; $v = 10^{-7}$.

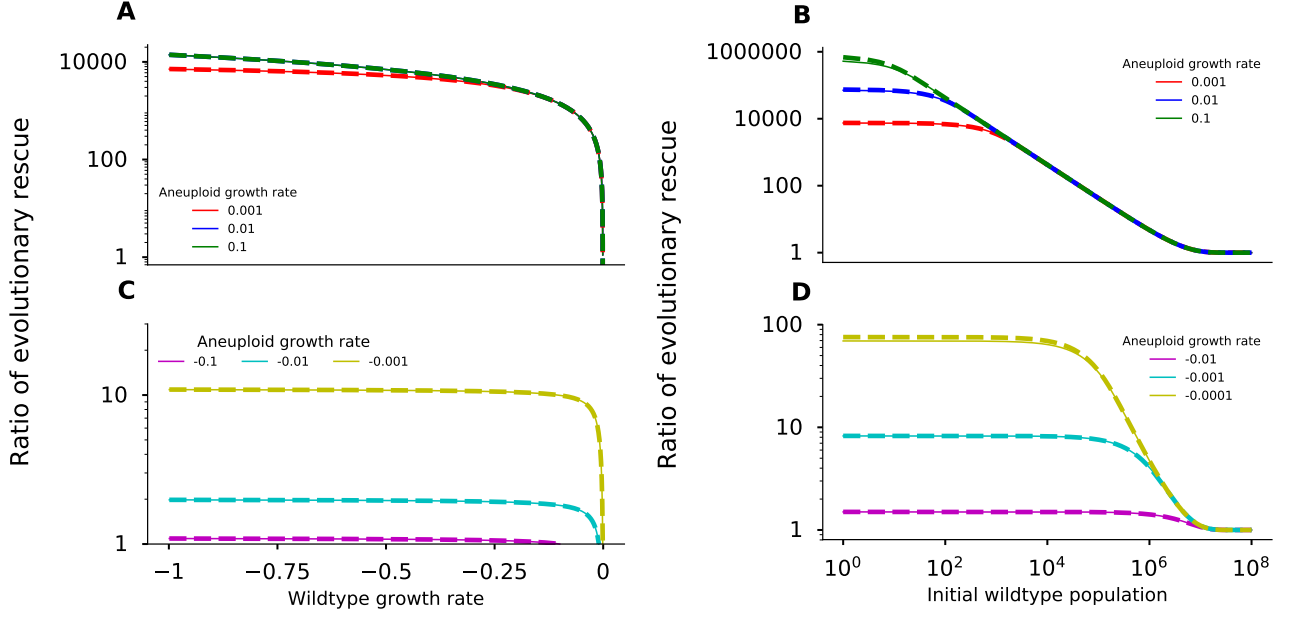


Figure 6: Effect of aneuploidy on evolutionary rescue. The ratio of rescue probability with and without aneuploid (H , ??) increases with the aneuploid growth rate (colors) and decreases with the wildtype growth rates and initial tumor size (x-axes), except for large tumors where the ratio converges to unity. (A, B) Aneuploidy provides partial resistance. (C, D) Aneuploidy provides tolerance. Solid and dashed lines apply p_{rescue} from the exact formula of (eq. (11) in eq. (2)); dashed lines apply p_{rescue} from the approximate formula (eq. (23)), with good agreement. Parameters: $N = 10^4$; $\lambda_w = \lambda_a = \lambda_m = 0.14$; (B) $\mu_w = 0.17$; $\mu_m = 0.13$; $u = 10^{-2}$; $v = 10^{-7}$.

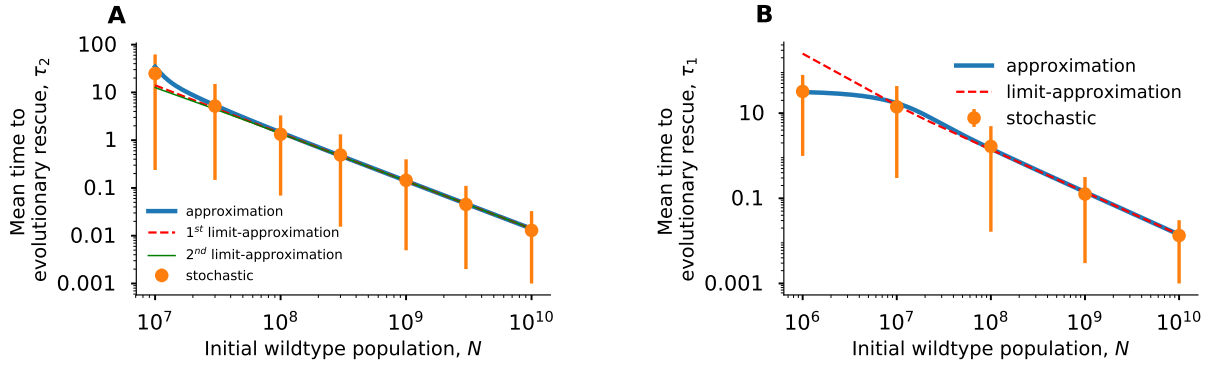


Figure 7: Evolutionary rescue time. Shown is the mean time for appearance of a resistance mutation the leads to evolutionary rescue (left) with ($u > 0$) and (right) without ($u = 0$ aneuploidy). Our inhomogeneous Poisson-process approximations (solid blue lines, right: eq. (25), left: eq. (31)) is in agreement with simulation results (orange markers with 95% CI). Our 1st-order (dashed red lines, right: eq. (7), left: ??) and 2nd-order (green line, left: ??) approximations work well when the initial tumor size is large (here $> 10^8$ cells). Parameters: $\lambda_w = \lambda_a = \lambda_m = 0.14$; $\mu_w = 0.17$; (A) $\mu_a = 0.145$; $\mu_m = 0.13$; $u = 10^{-2}$; $v = 10^{-7}$.

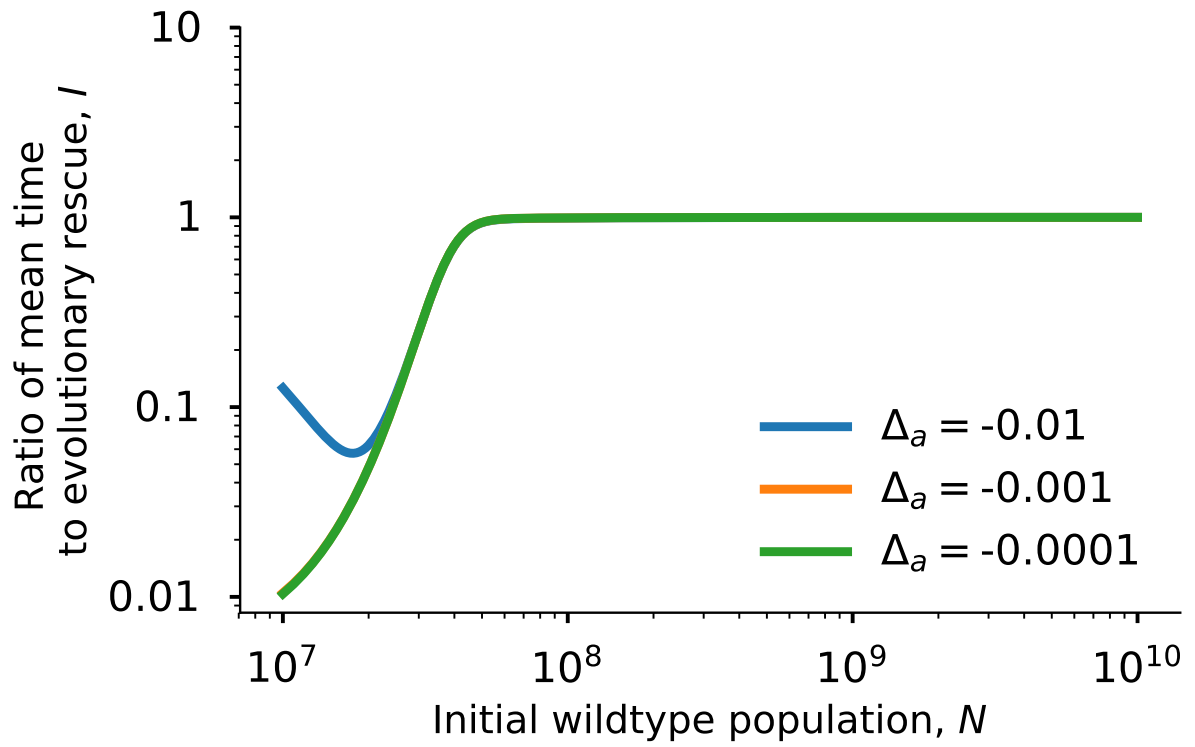


Figure 8: Ratio of evolutionary rescue time with and without aneuploidy. The ratio of the mean time to appearance of a resistance mutation that leads to evolutionary rescue with ($u > 0$) and without ($u = 0$) aneuploidy for variable initial tumor sizes (eq. (9)) when aneuploidy provides tolerance to the drug ($\Delta_a < 0$). When the initial tumor size is not large ($< 10^8$), aneuploidy can decrease the rescue time by 10-100-fold.

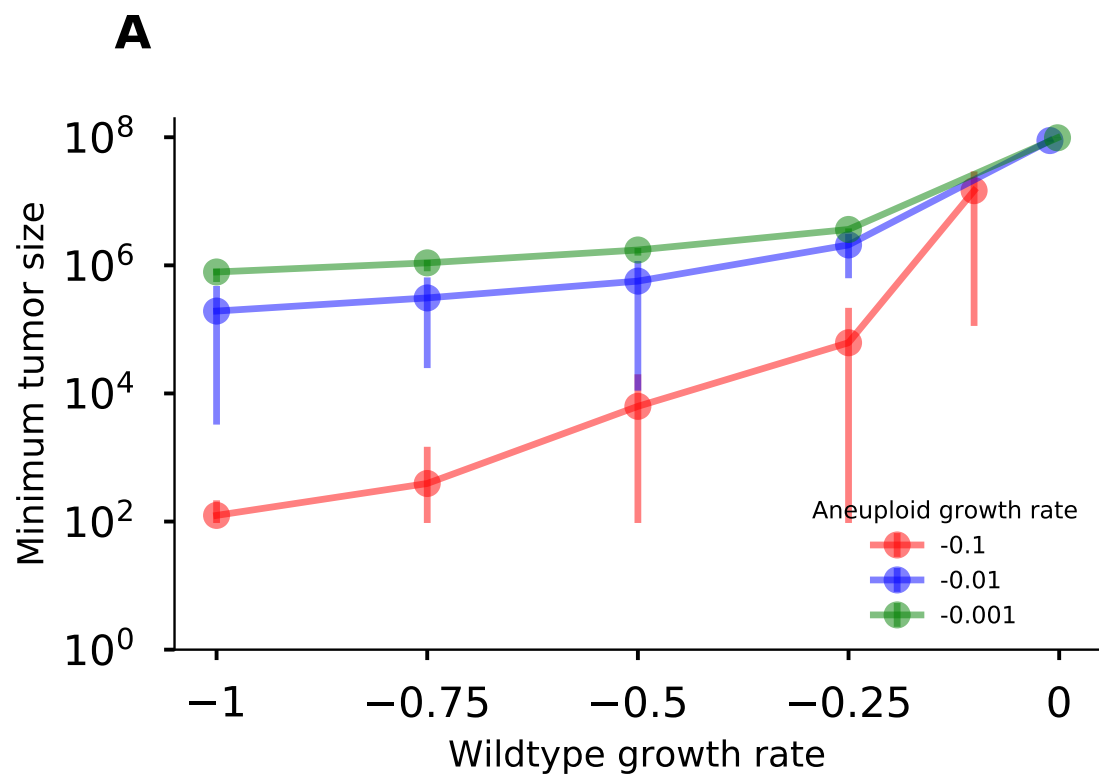


Figure 9: TODO. TODO

Appendices

Appendix A: Survival probability of a single lineage

To analyze evolutionary rescue in this model, we use the framework of *multitype branching processes* (Harris et al., 1963, ?). This allows us to find explicit expressions for the *survival probability*: the probability that a lineage descended from a single cell does not become extinct.

Let p_w , p_a , and p_m be the survival probabilities of a population consisting initially of single wildtype cell, aneuploid cell, or mutant cell, respectively. The complements $1 - p_w$, $1 - p_a$, and $1 - p_m$ are the extinction probabilities, which satisfy each its respective equation,

$$\begin{aligned} 1 - p_w &= \frac{\mu_w}{\lambda_w + \mu_w + u + v} + \frac{u}{\lambda_w + \mu_w + u + v} (1 - p_a) + \\ &\quad \frac{\lambda_w}{\lambda_w + \mu_w + u + v} (1 - p_w)^2 + \frac{v}{\lambda_w + \mu_w + u + v} (1 - p_m), \\ 1 - p_a &= \frac{\mu_a}{\lambda_a + \mu_a + v} + \frac{v}{\lambda_a + \mu_a + v} (1 - p_m) + \frac{\lambda_a}{\lambda_a + \mu_a + v} (1 - p_a)^2, \\ 1 - p_m &= \frac{\mu_m}{\lambda_m + \mu_m} + \frac{\lambda_m}{\lambda_m + \mu_m} (1 - p_m)^2. \end{aligned} \tag{10}$$

The survival probabilities are given by the smallest solution for each quadratic equation (Uecker et al., 2015). Therefore we have

$$\begin{aligned} p_w &= \frac{\lambda_w - \mu_w - u - v + \sqrt{(\lambda_w - \mu_w - u - v)^2 + 4\lambda_w(u p_a + v p_m)}}{2\lambda_w}, \\ p_a &= \frac{\lambda_a - \mu_a - v + \sqrt{(\lambda_a - \mu_a - v)^2 + 4\lambda_a v p_m}}{2\lambda_a}, \\ p_m &= \frac{\lambda_m - \mu_m}{\lambda_m}. \end{aligned} \tag{11}$$

Note that the equation for p_w depends on both p_a and p_m , and the equation for p_a depends on p_m . To proceed, we can plug the solution for p_m and p_a into the solution for p_w . We perform this for three different scenarios.

Scenario 1: Aneuploid cells are partially resistant

We first assume that aneuploidy provides partial resistance to drug therapy, $\lambda_a > \mu_a$, and that this resistance is significant, $(\lambda_a - \mu_a - v)^2 > 4\lambda_a v p_m$. We thus rewrite eq. (11) as

$$\begin{aligned} p_w &= \frac{\lambda_w - \mu_w - u - v}{2\lambda_w} \left(1 - \sqrt{1 + \frac{4\lambda_w(v p_m + u p_a)}{(\lambda_w - \mu_w - u - v)^2}} \right), \text{ and} \\ p_a &= \frac{\lambda_a - \mu_a - v}{2\lambda_a} \left(1 + \sqrt{1 + \frac{4\lambda_a v p_m}{(\lambda_a - \mu_a - v)^2}} \right). \end{aligned}$$

Using the quadratic Taylor expansion $\sqrt{1+x} = 1 + x/2 + O(x^2)$ and assuming $u, v \ll 1$, we obtain the following approximation for the survival probability of a population initially consisting of a single

wildtype cell,

$$p_w \approx -\frac{vp_m + up_a}{\lambda_w - \mu_w - u - v} \quad (12)$$

$$\approx -\frac{1}{\lambda_w - \mu_w} \left[\frac{u(\lambda_a - \mu_a)}{\lambda_a} + \frac{uv(\lambda_m - \mu_m)}{\lambda_m(\lambda_a - \mu_a)} + \frac{v(\lambda_m - \mu_m)}{\lambda_m} \right] \quad (13)$$

Now uv is very small, and if we assume $v \ll u$, we have

$$p_w \approx \frac{u}{|\Delta_w|} \cdot \frac{\Delta_a}{\lambda_a}. \quad (14)$$

Second-order approximation. To improve our approximation, we can consider the second term of the Taylor series expansion,

$$\left(1 + \frac{4\lambda_a vp_m}{(\lambda_a - \mu_a - v)^2} \right)^{\frac{1}{2}} = 1 + \frac{2\lambda_a vp_m}{(\lambda_a - \mu_a - v)^2} - \frac{(\lambda_a vp_m)^2}{4(\lambda_a - \mu_a - v)^4} + \dots,$$

which gives us the following approximation,

$$p_a \approx \frac{\lambda_a - \mu_a - v}{\lambda_a} + \frac{vp_m}{\lambda_a - \mu_a - v} - \frac{\lambda_a (vp_m)^2}{8(\lambda_a - \mu_a - v)^3}. \quad (15)$$

We therefore have

$$\begin{aligned} p_w &\approx -\frac{1}{\lambda_w - \mu_w - u - v} \left[\frac{u(\lambda_a - \mu_a - v)}{\lambda_a} + \frac{uv(\lambda_m - \mu_m)}{\lambda_m(\lambda_a - \mu_a - v)} + \frac{v(\lambda_m - \mu_m)}{\lambda_m} - \frac{uv^2\lambda_a(\lambda_m - \mu_m)^2}{8\lambda_m^2(\lambda_a - \mu_a - v)^3} \right] \\ &\approx -\frac{1}{\lambda_w - \mu_w} \left[\frac{u(\lambda_a - \mu_a)}{\lambda_a} + \frac{uv(\lambda_m - \mu_m)}{\lambda_m(\lambda_a - \mu_a)} + \frac{v(\lambda_m - \mu_m)}{\lambda_m} - \frac{uv^2\lambda_a(\lambda_m - \mu_m)^2}{8\lambda_m^2(\lambda_a - \mu_a)^3} \right], \end{aligned} \quad (16)$$

and using $\Delta_k = \lambda_k - \mu_k$, we can write the above equation as

$$p_w \approx -\frac{1}{\Delta_w} \left(\frac{u\Delta_a}{\lambda_a} + \frac{uv\Delta_m}{\lambda_m\Delta_a} + \frac{v\Delta_m}{\lambda_m} - \frac{uv^2\lambda_a\Delta_m^2}{8\lambda_m^2\Delta_a^3} \right). \quad (17)$$

Scenario 2: Aneuploid cells are tolerant.

We now assume that aneuploidy provides tolerance to drug therapy, that is, the number of aneuploid cells significantly declines over time, but at a lower rate than the number of wildtype cells, $\lambda_w - \mu_w < \lambda_a - \mu_a < 0$. We also assume that the decline are significant, $(\lambda_a - \mu_a - v)^2 > 4\lambda_a vp_m$. We rewrite eq. (11) as

$$\begin{aligned} p_w &= \frac{\lambda_w - \mu_w - u - v}{2\lambda_w} \left(1 - \sqrt{1 + \frac{4\lambda_w (vp_m + up_a)}{(\lambda_w - \mu_w - u - v)^2}} \right), \text{ and} \\ p_a &= \frac{\lambda_a - \mu_a - v}{2\lambda_a} \left(1 - \sqrt{1 + \frac{4\lambda_a vp_m}{(\lambda_a - \mu_a - v)^2}} \right). \end{aligned} \quad (18)$$

Since $u, v \ll 1$, the term in the root can be approximated using a 1st-order Taylor expansion. So, substituting the expressions for p_a and p_m , we have

$$\begin{aligned}
p_w &\approx -\frac{vp_m + up_a}{\lambda_w - \mu_w - u - v} \\
&\approx \frac{1}{\lambda_w - \mu_w - u - v} \left[\frac{uv(\lambda_m - \mu_m)}{\lambda_m(\lambda_a - \mu_a - v)} - \frac{v(\lambda_m - \mu_m)}{\lambda_m} \right] \\
&\approx \frac{v(\lambda_m - \mu_m)}{\lambda_m(\lambda_w - \mu_w)} \left[\frac{u}{(\lambda_a - \mu_a)} - 1 \right] \\
&= \frac{v\Delta_m}{\lambda_m|\Delta_w|} \left(\frac{u}{|\Delta_a|} + 1 \right).
\end{aligned} \tag{19}$$

Scenario 3: Aneuploid cells are non-growing

We now assume that the growth rate of aneuploid cells is close to zero (either positive or negative), such that $(\lambda_a - \mu_a - v)^2 < 4\lambda_a vp_m$. We rewrite eq. (11) as

$$p_a = \frac{\lambda_a - \mu_a - v + 2\sqrt{\lambda_a vp_m} \left(1 + \frac{(\lambda_a - \mu_a - v)^2}{4\lambda_a vp_m} \right)^{\frac{1}{2}}}{2\lambda_a}. \tag{20}$$

Using a following Taylor series expansion for small $(\lambda_a - \mu_a - v)^2 / 4\lambda_a vp_m$,

$$\left(1 + \frac{(\lambda_a - \mu_a - v)^2}{4\lambda_a vp_m} \right)^{\frac{1}{2}} = 1 + \frac{(\lambda_a - \mu_a - v)^2}{8\lambda_a vp_m} + \dots,$$

we obtain the approximation

$$\begin{aligned}
p_a &\approx \frac{\lambda_a - \mu_a - v + 2\sqrt{\lambda_a vp_m} \left[1 + \frac{(\lambda_a - \mu_a - v)^2}{8\lambda_a vp_m} \right]}{2\lambda_a} \\
&= \frac{\lambda_a - \mu_a - v + 2\sqrt{\lambda_a vp_m} + \frac{(\lambda_a - \mu_a - v)^2}{4\sqrt{\lambda_a vp_m}}}{2\lambda_a} \\
&= \frac{(\lambda_a - \mu_a - v + 2\sqrt{\lambda_a vp_m})^2 + 4\lambda_a vp_m}{8\lambda_a \sqrt{\lambda_a vp_m}} \\
&= \frac{4\lambda_a vp_m + 4\lambda_a vp_m \left(1 + \frac{\lambda_a - \mu_a - v}{2\sqrt{\lambda_a vp_m}} \right)^2}{8\lambda_a \sqrt{\lambda_a vp_m}} \\
&= \frac{1}{2\lambda_a} \left(\lambda_a - \mu_a - v + 2\sqrt{\lambda_a vp_m} \right).
\end{aligned} \tag{21}$$

Plugging this in eq. (19), the survival probability of a population starting from one wildtype individual is

$$\begin{aligned}
p_w &\approx -\frac{1}{\lambda_w - \mu_w - u - v} \left[v \frac{\lambda_m - \mu_m}{\lambda_m} + \frac{u}{2\lambda_a} \left(\lambda_a - \mu_a - v + 2\sqrt{\lambda_a vp_m} \right) \right] \\
&= -\frac{1}{\lambda_w - \mu_w - u - v} \left[v \frac{\lambda_m - \mu_m}{\lambda_m} + \frac{u}{2\lambda_a} (\lambda_a - \mu_a - v) + u \sqrt{\frac{v(\lambda_m - \mu_m)}{\lambda_a \lambda_m}} \right].
\end{aligned} \tag{22}$$

Appendix B: Evolutionary rescue probability

Substituting eqs. (12), (19) and (22) into eq. (2), the evolutionary rescue probability can be approximated by

$$p_{\text{rescue}} \approx \begin{cases} 1 - \exp \left[\frac{N}{\Delta_w - u - v} \left(v \frac{\Delta_m}{\lambda_m} + \frac{u(\Delta_a - v)}{2\lambda_a} + u \sqrt{\frac{v\Delta_m}{\lambda_a \lambda_m}} \right) \right], & 4\lambda_a v p_m > (\Delta_a - v)^2, \\ 1 - \exp \left[\frac{v\Delta_m N}{\lambda_m \Delta_w} \left(1 - \frac{u}{\Delta_a} \right) \right], & \Delta_a < 0 \quad \text{and} \quad 4\lambda_a v p_m < (\Delta_a - v)^2, \\ 1 - \exp \left[\frac{N}{\Delta_w} \left(\frac{u\Delta_a}{\lambda_a} + \frac{uv\Delta_m}{\lambda_m \Delta_a} + \frac{v\Delta_m}{\lambda_m} \right) \right], & \Delta_a > 0 \quad \text{and} \quad 4\lambda_a v p_m < (\Delta_a - v)^2. \end{cases} \quad (23)$$

Appendix C: Evolutionary rescue time

We first calculate the expected time for the appearance of the first mutant that rescues the cell population. This can occur either through the evolutionary trajectory *wildtype* \rightarrow *mutant* or through the trajectory *wildtype* \rightarrow *aneuploid* \rightarrow *mutant*. We start with the former.

Assuming no aneuploidy ($u = 0$), we define T_1 to be the time at which the first mutant cell appears that will avoid extinction and will therefore rescue the population. Note that if extinction occurs, that is the frequency of mutants after a very long time is zero, $m_\infty = 0$, then it is implied that $T_1 = \infty$, and vice versa if $T_1 < \infty$ then $m_\infty > 0$.

The number of successful mutants generated until time t can be approximated by an inhomogeneous Poisson process with rate $R(t) = u p_a w_t$, where $w_t = N e^{\Delta_w t}$ is the number of wildtype cells at time t . Note that

$$\int_0^t R(z) dz = u p_a N \frac{\exp[\Delta_w t] - 1}{\Delta_w} \approx u p_a N t, \quad (24)$$

by integrating the exponential and because $\frac{\exp[\Delta_w t] - 1}{\Delta_w} = \frac{1 + \Delta_w t + O(t^2) - 1}{\Delta_w} = t + O(t^2)$. The probability density function of T_1 is thus $R(t) \exp\left(-\int_0^t R(z) dz\right)$. Therefore, the probability density function of the conditional random variable ($T_1 \mid T_1 < \infty$) is $f_1(t) = \frac{R(t) \exp\left(-\int_0^t R(z) dz\right)}{p_{\text{rescue}}}$.

We are interested in the mean conditional time, $\tau_1 = \mathbb{E}[T_1 \mid T_1 < \infty]$, which is given by

$$\tau_1 = \int_0^\infty t f_1(t) dt = \frac{\int_0^\infty t R(t) \exp\left(-\int_0^t R(z) dz\right) dt}{p_{\text{rescue}}} = \frac{\int_0^\infty \exp\left(-\int_0^t R(z) dz\right) dt}{p_{\text{rescue}}} \quad (25)$$

after applying integration by parts. Therefore, plugging eqs. (24) and (2) in eq. (25),

$$\tau_1 = \frac{\int_0^\infty e^{-u N p_a \frac{e^{\Delta_w t} - 1}{\Delta_w}} dt}{1 - (1 - p_w)^N} \approx \frac{\int_0^\infty \exp(-u p_a N t) dt}{1 - e^{-N p_w}} \approx \quad (26)$$

$$\left(1 + e^{-N p_w}\right) \int_0^\infty e^{-u p_a N t} dt = \frac{1 + e^{-N p_w}}{u p_a N}, \quad (27)$$

where we use the approximations $\frac{e^{\Delta_w t} - 1}{\Delta_w} = \frac{1 + \Delta_w t + O(t^2) - 1}{\Delta_w} = t + O(t^2)$ and $(1 - e^{-N p_w})^{-1} \approx 1 + e^{-N p_w}$ and integrate the exponent. Figure 7B show the agreement between this approximating and simulation results for intermediate and large tumor sizes.

When $Nu \gg 1$ the aneuploid frequency dynamics is roughly deterministic and therefore can be approximated by

$$a_t \approx \frac{Nue^{\Delta_w t}}{\Delta_w - \Delta_a} \left[1 - e^{(\Delta_w - \Delta_a)t} \right]. \quad (28)$$

As a result, when $N \gg 1$ the number of successful mutants created by direct mutation and via aneuploidy can be approximated by inhomogeneous Poisson processes with the rates

$$r_1(t) = v p_m \int_0^t a_z dz = \frac{uvNp_m}{\Delta_w - \Delta_a} \left(\frac{e^{\Delta_w t} - 1}{\Delta_w} - \frac{e^{\Delta_a t} - 1}{\Delta_a} \right), \quad (29)$$

$$r_2(t) = v p_m \int_0^t w_z dz = vNp_m \frac{e^{\Delta_w t} - 1}{\Delta_w}. \quad (30)$$

For large initial population sizes we assume that the two processes are independent and as a result, they can be merged into a single Poisson process with rate $(r_1 + r_2)(t)$. Consequently, the mean time to the appearance of the first rescue mutant is

$$\tau_2 = \frac{\int_0^\infty e^{-(r_1(t)+r_2(t))} dt}{1 - (1 - p_w)^N} = \frac{\int_0^\infty \exp \left[-\frac{uvNp_m}{\Delta_w - \Delta_a} \left(\frac{e^{\Delta_w t} - 1}{\Delta_w} - \frac{e^{\Delta_a t} - 1}{\Delta_a} \right) - vNp_m \frac{e^{\Delta_w t} - 1}{\Delta_w} \right] dt}{1 - (1 - p_w)^N}, \quad (31)$$

which we plot in Figure 7A as a function of the initial population size, N .

We wish to obtain a simpler formula for τ_2 , similar to eq. (7). We thus have the following expansions,

$$\begin{aligned} \frac{e^{\Delta_w t} - 1}{\Delta_w} &= \frac{1 + \Delta_w t + O(t^2) - 1}{\Delta_w} = t + O(t^2), \\ \frac{e^{\Delta_a t} - 1}{\Delta_a} &= \frac{1 + \Delta_a t + O(t^2) - 1}{\Delta_a} = t + O(t^2), \end{aligned}$$

which we use to derive a first-order approximation for τ_2 ,

$$\tau_2 \approx \left(1 + e^{-Np_w} \right) \int_0^\infty e^{-uNp_m t} dt = \frac{(1 + e^{-Np_w})}{uNp_m}, \quad (32)$$