Masking, maintenance and mimicry: the interplay of cell-intrinsic and cell-extrinsic effects in evolutionary games

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ABSTRACT

Evolution underpins the survival of a population under environmental pressure. Resistance to treatment commonly arises as a result of such evolution. We analytically examine the addition of frequency-dependent effects on evolutionary outcomes. Through the lens of experimental biology, we frame these interactions as cell-extrinsic, growth rate-modifying, ecological interactions. Additionally, we show the extent to which the presence of these ecological interactions can modify evolutionary trajectories predicted from cell-intrinsic properties alone and show that these interactions can modify evolution in such ways as to mask or mimic or maintain the results of cell-intrinsic fitness advantages. This work has implications for the interpretation and understanding of evolution, a result which may explain an abundance of apparently neutral evolution in cancer systems and similarly heterogeneous populations. In addition, the derivation of an analytical result for stochastic, ecologically dependent evolution paves the way for treatment approaches involving genetic and ecological control.

Author Summary

Through analytical and simulation methods we focus on decomposing the cell-intrinsic and cell-extrinsic interactions in a game-theoretic framework for interacting subpopulations in a genetic system. We highlight the ability of extrinsic contributions to arbitrarily alter the evolution of a population of interacting agents. We derive an exact solution to the 1-dimensional Fokker-Planck equation for a two-player genetic system including mutation, selection, drift and games. Examining how the strength of the specific game interactions alters our analytical solution, we validate these theoretical predictions in simulations. We derive expressions for the conditions on the game interactions in this one-dimensional case that mask the cell-intrinsic monoculture landscape dynamics.

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Introduction

It has been observed across many systems that populations of cells, especially under heavy selection pressure, must adapt to survive. This Darwinian survival of the fittest results in the selection of mutations and a changing frequency of observed genotypes in a population[1]. Sufficiently strong evolutionary pressure can alter the genotypes of the entire population over subsequent generations. Traditionally, this has been considered a result of the intrinsic fitness of each genotype under the external evolutionary pressure of the environment, whether that is drug, temperature or other. In this regard, the genetic fitness landscape has been one method of understanding this cell-intrinsic fitness, selection and evolution. Fitness landscapes map out the fitness or reproductive potential of an individual based on its particular genotype location in genotype space. Peaks in this landscape correspond to high fitness or reproductive potential. Over time, populations *migrate* to local peaks in the landscape where they reproduce more quickly than at their initial location. The initial location, thereby, corresponds to a less "fit" genotype. Landscapes also allow for an understanding of neutral evolution; in this formalism flat regions of a fitness landscape are equally fit and thus all movement is through diffusive neutral evolution, increasing evolutionary stochasticity[2].

It is a frequent assumption that intrinsic cell fitness determines selection and evolutionary progression[3]. This assumption also means that it is common to predict evolution of a population based upon observed fitnesses of cells under treatment in mono-culture[4]. This assumption also extends to the belief that genotypic fitness is the explanatory variable when we analyse

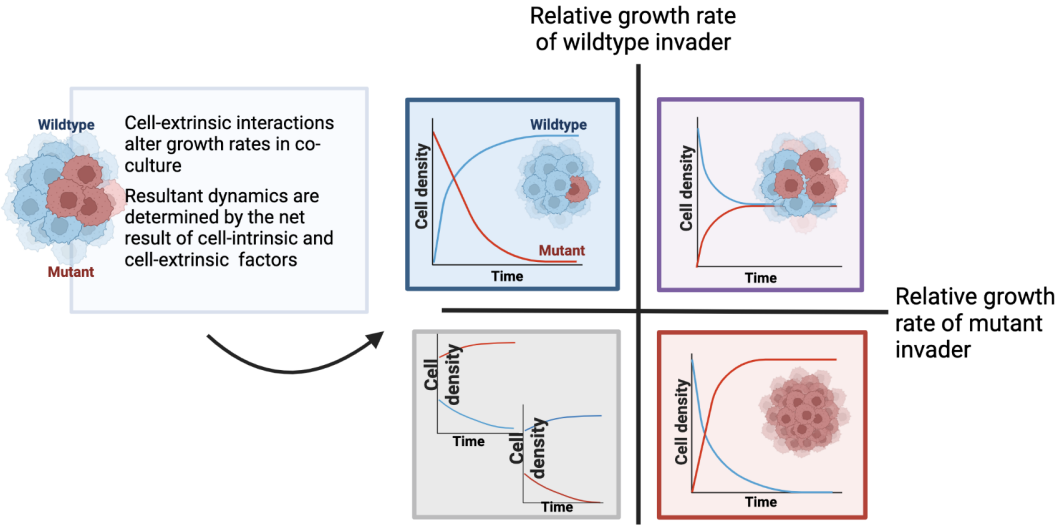

Figure 1. Illustration of game dynamics between two populations (wildtype and mutant cells). Evolutionary game theory between two players results in four types of dynamics. In the biological case, both the cell-intrinsic and cell-extrinsic influences on growth rate come together in co-culture to produce novel dynamics. The observed dynamics rely not only on the isolated behavior of cells, but also on the precise balance between intrinsic and extrinsic factors. In the top left quadrant, the wildtype cell type dominates. In the top right quadrant, a heterogeneous mixture is promoted. In the bottom right quadrant, the resistant mutant cell type dominates. Lastly, in the bottom left quadrant, coexistence is unstable and the populations are driven to the nearest stable fixed point (all wildtype or all mutant).

the evolutionary trajectory of genotypes in experiments. In spite of the development of many effective targeted drugs, it is often the case that personalized treatment and drug development techniques eventually encounter failure. One possible explanation for this discrepancy lies in the problem of tumor heterogeneity and that in addition to the genetic selection of genotypes, the interactions between cell populations can also impact the fitness of a population[5,6]. Game theoretic interactions are added to a model to reflect the presence of population frequency dependence in the fitness of an individual. When these ideas are incorporated into evolutionary models, the result is frequency-dependent selection(**Figure 1**).

The presence of frequency-dependent selection has been observed to be a mechanism for maintaining diversity, including observations within experimental bacterial systems[7–12]. Theoretical studies have also shown the potential of frequency-dependent selection to promote high mutation rates and to accelerate evolution[13,14]. Frequency-dependent selection has also been observed in cancer cell lines and in varying microenvironments; Kaznatcheev *et al.*[15] demonstrated how alectinib-resistant and parental non-small cell lung cancer cells have different fitnesses in different relative population frequencies and how the presence of fibroblasts or changing treatment results in different evolutionary games. Many papers have also used game theory to model tumour growth and composition with the presence of game interactions between cells including the interaction of competing tumor and stromal cells and the production of growth factors as a strategy[16–22]. An underlying assumption of many of these models, including ours, is that the strategy of a cell is set by its genotype and therefore by its parent. Under this assumption, the "payoff" of the evolutionary game is reflected in a cell's ability to replicate, i.e. producing another player of the same type.

Frequency-dependent selection has been modelled in multiple ways, one of which is a frequency-dependent Moran process[23]. We focus on the more common Wright-Fisher formulation, expanding upon multiple works that have formalised the frequency-dependent Wright-Fisher model in multiple dimensions[24,25]. The fixation time of the Wright-Fisher model and associated conservation laws have also been derived[26,27].

We hypothesise that in the frequency-dependent Wright-Fisher model, game interactions can even fully mask or mimic genetic fitness, producing population dynamics that are not reflective of the ranking of cell-intrinsic fitnesses within the genetic landscape alone(**Figure 1**). Using a stochastic, agent-based model, we simulate a frequency-dependent Wright-Fisher model in 1-dimension. We derive and validate expressions for the conditions on the game interactions in the 1-dimensional case that cause the resultant evolution to maintain, mimic or mask the dynamics of an initial genetic fitness landscape. The conclusion of such a hypothesis is that when ecological factors and interactions change the fitness of cells, the evolution of the population can only be modelled using game theory and population genetics in tandem. Our work emphasises that knowledge of cell-extrinsic interaction strengths is essential in accurately predicting evolution in co-culture.

Results

Whilst monoculture growth rates of various cell lines are frequently measured, particularly under various selective pressures such as drugs, the properties of these same cell types whilst interacting in co-cultures are less frequently measured and understood. We develop both deterministic and stochastic mathematical approaches to study the evolutionary games in a genetic population under the assumption that the cell-intrinsic growth rates are modified by interaction with another population, also using computational approaches to model the impact of varying only interaction terms. We examine, predominantly, the dynamics of a two-player system, with strategies we term wildtype and mutant to reflect resistance evolution and consider the evolutionary dynamics of this type of interacting population both with and without noise.

Deterministic evolutionary game theory

Game theory is the study of the dynamics which result from the interaction of different strategies played against each other. Specific strategies result in expected payoffs for the players and thus the average payoff depends upon the frequency of strategies in the system. Differential game theory can describe deterministic solutions for these dynamics by means of differential equations. In a symmetric game, the time ordering of played strategies does not effect the payoffs for the players involved. The payoffs in a 2-strategy symmetric differential game can be presented as a payoff matrix in the following way:

$$P = \begin{pmatrix} a & b \\ c & d \end{pmatrix} \tag{1}$$

where a is the payoff for a player with strategy 1 playing against another player of type 1, b is the payoff for a player with strategy 1 playing against a player of type 2, c is the payoff for a player with strategy 2 playing against a player of type 1, and d is the payoff for a player with strategy 2 playing against a player of type 2. These values determine the expected payoffs or "fitness" for each player when the frequencies of strategies in the population is known.

Within biology, available "strategies" are the growth rates of the populations under study. In this case, a cell, for example, may not have the ability to actively choose a strategy. Under the assumption that a cell's genome determines its strategy we use an abstract model of the genome comprising a single site of interest, two alleles are possible, the wildtype with no mutation in the relevant gene and the mutant, harbouring a resistance mutation at the site of interest. In genetic population models, strategy proportions change when cells undergo self-replication, with fitter strategies reproducing at a faster rate. In this type of model, the cells are called replicators; players with strategies that are determined by the strategy of their parent. The fitness of a cell's strategy determines its rate of replication and this formalism and accompanying equations are referred to as replicator dynamics. The replicator equation describes the dynamics of the i^{th} population fraction x_i as a function of the payoff matrix P (Eq. 12) where the payoff matrix, P contains the fitness information of each cell type (strategy). In the 2-dimensional case with payoff matrix (**Eq. 1**), this reduces to the following form;

$$\dot{x} = x(1-x)((1-x)(b-d) - x(c-a)), \tag{2}$$

where the proportion of type 2 is $1-x$. Without the addition of noise, the replicator equation gives us deterministic solutions for the evolutionary stable strategies present. Without mutation in the population, this equation has solutions at $x = 0$, $x = 1$ and $x = (b-d)/((b-d)+(c-a))$. As such different conditions on a, b, c and d result in different types of evolutionary stable solutions. These different conditions are often labelled as different types of games (hawk-dove, snowdrift etc.). One can construct the 2-dimensional "game space", which has the axes $c-a$ and $b-d$ as shown in **Figure 1**. A game's position in game space categorizes it into a certain universality class, for example in the 2-dimensional "game space" multiple games in the same quadrant may generate similar dynamics.

Cell-intrinsic and cell-extrinsic components can be logically separated within the payoff matrix

In the payoff matrix for two genetically distinct cell populations, the diagonal terms, a and d represent the monoculture growth rates of each population. To illustrate and examine this, we modify the traditional payoff matrix, P, and decompose the cell-intrinsic growth rates from the co-culture effects as follows;

$$P = \begin{pmatrix} a & b \\ c & d \end{pmatrix} = a \begin{pmatrix} 1 & 1+\alpha_{mw} \\ 1+s_m+\alpha_{wm} & 1+s_m \end{pmatrix}, \tag{3}$$

where s_m is the selective advantage of the mutant cell and a_{ij} is the growth-normalised interaction effect of population j on population i. The value of a experimentally is the replication rate of the wildtype cell, where the replication rate of the mutant can be represented by d or $a(1+s_m)$.

When there are no interactions ($\alpha_{wm},\ \alpha_{mw} \to 0$), there is no frequency dependence, the rows of the payoff matrix become constant and the differential equations become uncoupled. In this case the deterministic non-interacting evolutionary dynamics

are recapitulated. We denoted the normalized game modifications to the growth rates in this system with mutant and wildtype cells as α_{wm} and α_{mw}. Thus any relative dynamics in a two-player system can be defined by game coefficients α_{wm}, α_{mw} and selection coefficient s_m.

Transformation reveals interaction dependency of the selection coefficient

Game dynamics between wildtype and mutant alleles result in frequency-dependent selection with a dependence on both the intrinsic growth rates of the cells and the interactions α_{wm} and α_{mw}. As the presence of interactions alters the resultant dynamics, we introduce the idea of the effective selection coefficient σ_m. In the absence of stochastic effects, the resultant fitness advantage of the mutant, $\sigma_m(x)$, over the wildtype becomes

$$\sigma_m(x) = \frac{s_m - \alpha_{wm} + (\alpha_{wm} + \alpha_{mw})x}{1 + \alpha_{wm} - \alpha_{wm}x} \tag{4}$$

, where s_m is the intrinsic growth advantage of the mutant, x is the proportion of wildtype cells in the population, $\phi_m(x)$ is the fitness of the mutant and $\phi_w(x)$ is the frequency dependent fitness of the wildtype.

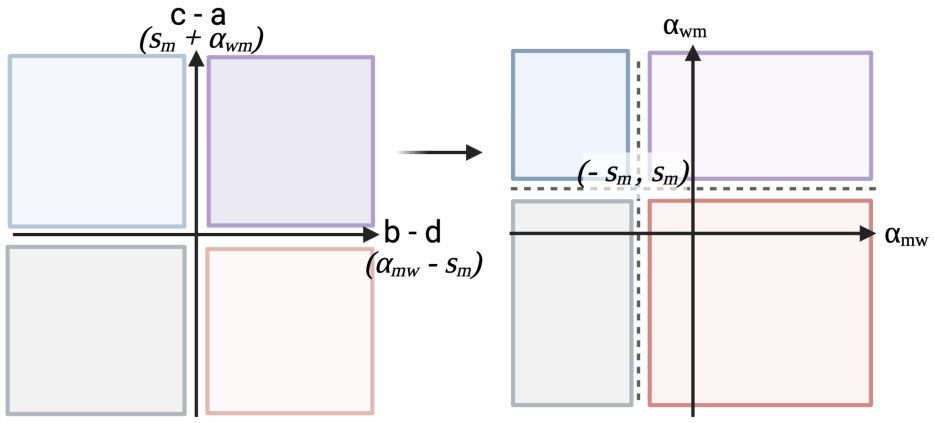

Figure 2. When viewed in interaction space, the classical quadrants are transformed by the magnitude of the mutant selection advantage Under the transformation to interaction space under a given selection advantage s_m the axes are the interaction term α_{mw}, α_{wm} and the boundaries between the dynamics in the game space quadrants are translated by the mutant selection advantage such that the critical point occurs at $(-s_m, s_m)$. The color of the quadrants and subsequent regions in the transformed resultant dynamics plot refer to the different types of evolutionary game dynamics illustrated in **Figure 1**

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The traditional quadrants in the game space for a two-player game correspond to different dynamical outcomes. We ask what these quadrant boundaries correspond to in terms of interaction strengths, and whether we can obtain their analytical forms. In this new basis the traditional game plot axes $c-a$ and $b-d$ become $a(\alpha_{mw} - s_m)$ and $a(s_m + \alpha_{wm})$ and the quadrants can be defined by conditions on α_{mw} and α_{wm} relative to the homogeneous mutant population selection coefficient s_m (**Figure 2**). The state space is shifted upwards and left by the selection coefficient s_m. As we move across the game-phase-space, the magnitudes of α_{wm} and α_{mw} relative to s_m and to each other can mean that the evolutionary outcome, the equilibrium distribution, can be modified, maintained or conversely the independent selection advantage masked.

Non-deterministic evolutionary dynamics

Although deterministic approaches to evolution can be useful, in real physical and biological systems stochastic fluctuations are present. In physical atomic systems, these fluctuations are typically due to heat, whereas within biological systems random genetic mutation and the inherently stochastic nature of replication both introduce biological noise. The Fokker-Planck equation[28,29] was originally derived to describe the time evolution of a particle undergoing both drag and brownian motion;

$$\frac{\partial \rho(x,t)}{\partial t} = -\frac{\partial}{\partial x}\Big[v(x)\rho(x,t)\Big] + \frac{\partial^2}{\partial x^2}\Big[D(x)\rho(x,t)\Big] \tag{5}$$

where $\rho(x,t)$ is the probability, at time t for a sub-population to make up fraction x of the population, $v(x)$ is the drift coefficient, and $D(x)$ is the diffusion coefficient. In general, the first term of this equation describes the evolution of a system under drift-like forces and the second term incorporates the random fluctuations. Versions of the Fokker-Planck equation, as a description

of evolution in time of a stochastic system, have been used successfully across physical and biological systems, including in descriptions of protein folding and gene expression stability[30–33] Kimura derived the appropriate form of the equation for genetic selection and diffusion via mutation and a general Gaussian solution to this equation[34].

Dynamics of an interacting population with noise

In order to find analytical solutions for this two-player genetic system with interactions and noise we look to find a solution to the Fokker-Planck formalism that includes the game interactions. As above we now understand how to write the wildtype and mutant fitness and thereby the payoff matrix in terms of interactions and selection coefficient s_m. One form of the steady-state solution of the Fokker-Planck equation is a Gaussian ansatz[34]. To use this method in the game context we assume that the selection coefficient in the potential function of the original ansatz is no longer $s_m x$ and is now a generic function $f(x)$ of the proportion, x, of the wildtype. We modify the potential, Φ, from the original Kimura solution and propose the population density function in the case of frequency dependence to be a normalised gaussian with modified potential.

Whilst the selection coefficient in the non-interacting version of the Fokker-Planck solution is just $s_m x$, we find that for the stationary distribution of the one-dimensional Fokker-Planck equation with noise from mutation and drift, a solution for the selection coefficient, $f(x)$ is given by:

$$f(x) = -2N\left[\frac{(s_m-\alpha_{wm})\alpha_{wm}+(\alpha_{wm}+\alpha_{mw})(1+\alpha_{wm})}{\alpha_{wm}^2}\ln(1+\alpha_{wm}-\alpha_{wm}x)+\frac{\alpha_{wm}+\alpha_{mw}}{\alpha_{wm}}x\right] \tag{6}$$

Therefore for any two-player system defined by game coefficients α_{mw}, α_{wm}, and selection coefficient s_m, we derive an analytic expression for an equilibrium solution for the population distribution $\rho(x)$ (**Figure 3**). We observe that at low mutation rates, this solution space is similar to the deterministic case in the absence of noise, whilst the values of both α_{mw} and α_{wm} and μN alter the width of the peaks in the probability distributions. The "snowdrift" game from the lower left quadrant in traditional game space is represented by the presence of two peaks in the Fokker-Planck distribution, the height of these peaks becomes more uneven with distance away from the line $\alpha_{wm} = \alpha_{mw} - 2s$ and the width of the peaks in all sections increase with mutation rate, μ.

Modification of the apparent selection coefficient

The result of these dynamics is such that the interaction terms can modify the dynamics that would be expected from monoculture information on growth rates alone. We consider the generic biological situation whereby the selective advantage of the mutant is modified. In this most general case of mapping, the independent original selection advantage of the mutant, s_m, is mapped in the interacting population to an effective selection σ_m, where both of the selection coefficients are greater than zero we equate the terms that determine the equilibrium distributions to find that α_{mw} must be equal to the following function of α_{wm}:

$$\alpha_{mw} = \frac{\alpha_{wm}(2\mu+\sigma_m-\sqrt{4\mu^2+\sigma_m^2})-\sigma_m(-2\mu-2s_m+\sigma_m+\sqrt{4\mu^2+\sigma_m^2})}{4\mu^2+(1+\sigma_m)(\sigma_m+\sqrt{4\mu^2+\sigma_m^2})-2\mu(1+\sigma_m+\sqrt{4\mu^2+\sigma_m^2})}. \tag{7}$$

Extrinsic interactions can mimic or mask cell-intrinsic selection advantages

It is possible that adding interaction to a neutral landscape mimics cell-intrinsic selection forces, with effective selection coefficient σ_m. We find that for added game interactions to create the appearance of such a landscape, we expand to examine higher order terms as $s_m \to 0$ and find that α_{wm} and α_{mw} in the off-diagonal terms must fulfil the criteria such that in the small mutation rate limit, this becomes

$$\alpha_{mw} = -\frac{\sigma_m}{1+\sigma_m}, \tag{8}$$

recovering the equivalent region of the deterministic game space that encapsulates this behavior.

Within cancer biology, we are particularly interested in the prevalence of neutral evolution, particularly in its ability to promote heterogeneity within a population[5]. Under neutral evolution, genetic sub-populations are interpreted to have no selective advantage. We ask under what conditions the presence of both cell-intrinsic selection advantages combined with game interactions can cause the evolutionary outcome of populations with non-zero intrinsic selection coefficients s_m to appear neutral[5]. In this case, intrinsic selection must be neutralized by interaction terms such that there is the absence of net selective effects ($\sigma_m = 0$). We find that added game interactions α_{wm} and α_{mw} must fulfil the following criteria in the small mutation rate limit;

$$\alpha_{mw} = \alpha_{wm} + 2s_m. \tag{9}$$

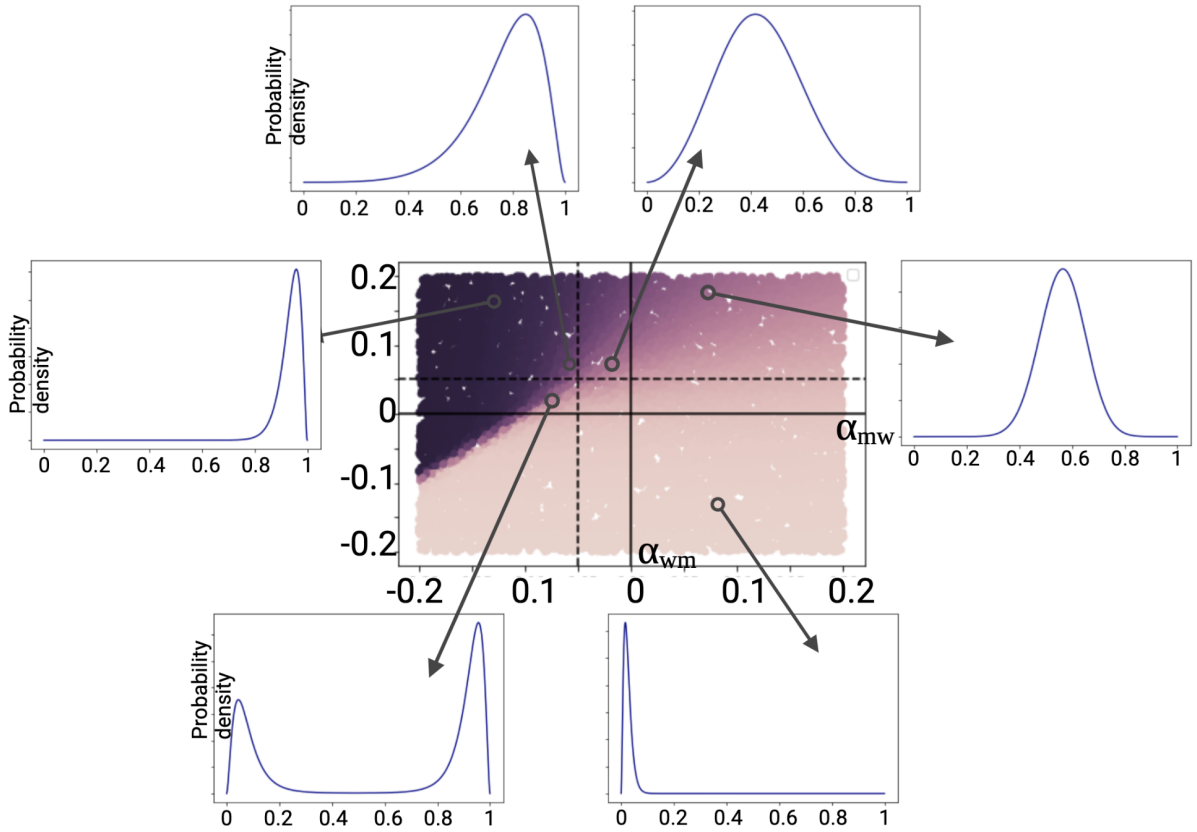

Figure 3. Fokker-Planck solution space for evolving population with stochastic fluctuation constant selection coefficient and varying interactions terms The central plot displays the mean value of the Fokker-Planck population probability distribution for varying interaction, with mutant selection advantage $s_m = 0.05$, $N = 1000$, $\mu = 0.005$. The average(mean) proportion of wildtype was plotted for each pair of interaction coefficients. 100% wildtype is dark purple, 0% wildtype population in cream. 10,000 random values of α_{mw}, α_{wm} in the interval $[-0.2, 0, 2]$ were sampled to populate the phase plot. Six specific examples of the analytical Fokker-Planck equilibrium solution are highlighted. The upper right quadrant shows a stable co-existence, with solutions in the bottom left quadrant representing probability distributions with two peaks, one at 100% wildtype and one at 0% wildtype.

In the game space, this is a line of possible solutions, meaning that there are technically infinitely many interaction possibilities that will appear neutral. We thus find conditions on our game coefficients such that we observe specific effects on our population distribution. These differ in high mutation limits from the boundaries in the deterministic results shown in **Figure 2**.

Maintaining the same evolutionary outcome under the presence of interactions

Biologically, whilst modification of selection may alter outcomes entirely, some cells, such as tumor cells, have evolved in such a way as to outgrow the healthy population fraction, whereas healthy cells optimise homeostatic development. Under evolution, successful malignant mutant cells must evolve to interact in such a way that the mutant cells are not extinguished under interactions and their selective advantage is maintained. Thus another key question of interest is to find the conditions under which a game with interaction strengths α_{mw}, α_{wm} and monoculture selection coefficient s_m maintain the same selective advantage and thus leave the evolutionary outcome unchanged, producing the same equilibrium distribution as if there were no interactions, $\sigma_m = s_m$. In the case that $s_m > 0$ we find that α_{wm} and α_{mw} must be related such that in the small mutation rate limit, $\mu^2 << 1$, when $\mu \Longrightarrow 0$ we regain the same restriction as from the deterministic form;

$$\alpha_{mw} = \frac{(\alpha_{wm} + \sigma_m)}{\sigma_m(1 + \sigma_m)}. \tag{10}$$

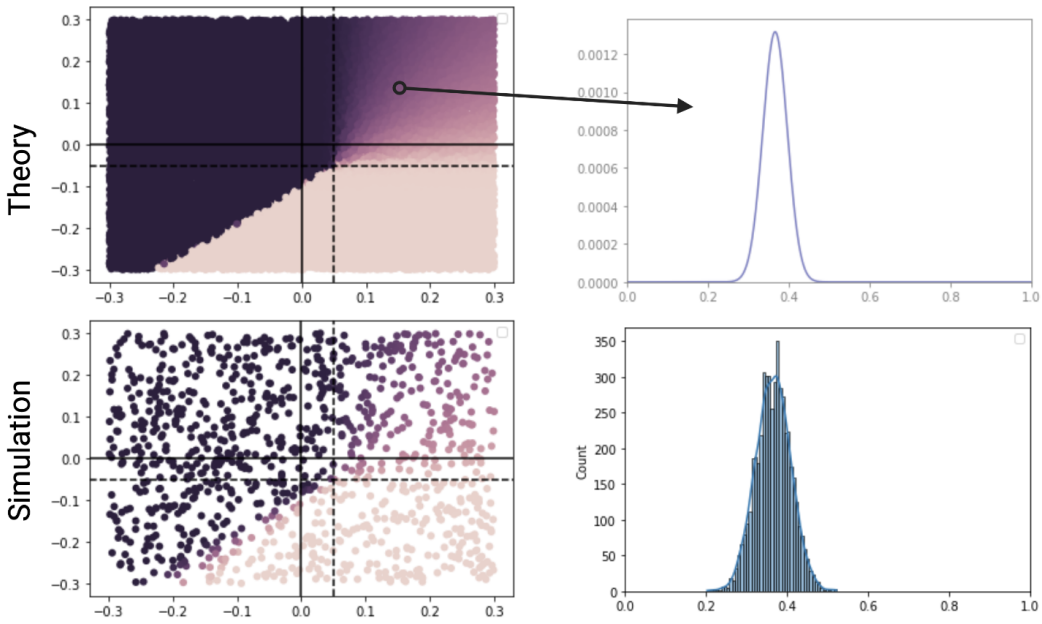

Figure 4. Fokker-Planck (FP) distribution compared to stochastic simulation results. The mean of the FP solution for $100,000$ different interaction strengths is shown (100% wildtype is dark purple and cream is 100% mutant allele fraction). Simulations are carried out and the results averaged for a subset of $1,000$ game coordinate pairs. We also look at the shape of the population density distribution in a specific Fokker-Planck solution compared to simulation. A histogram of $4,900$ simulations, at $1,000$ generations for specific game coordinates, is shown. We contrast also the analytical probability distribution in a specific game case, $\alpha_{wm} = 0.16,\ \alpha_{mw} = 0.14$, with a sampled distribution of the population fraction at $t = 1000$ generations from 1000 simulations under these conditions.

Stochastic simulations match Fokker-Planck under varying $s_m, \alpha_{mw}, \alpha_{wm}$

We wrote a stochastic, individual-based model to simulate a genetic population undergoing mutation and selection and to validate our equilibrium solutions of the genetic Fokker-Planck equation with interactions. For details see methods (code available at ...).

The Wright-Fisher model predicts that with mutation and selection, the population will move to the peak of the landscape in a single peaked landscape and in a flat landscape, will fluctuate stochastically around equal proportions of all genotypes. Traditionally, the results of the Wright-Fisher model depend strongly on the genetic fitness landscape it is based upon, in particular, whether the landscape is neutral or peaked. To explore the effect of games on evolutionary simulation models we added game interactions to a Wright-Fisher model on both single-peaked and flat landscapes. We simulated populations for $4,900$ random pairs of interaction coefficients (α_{mw}, α_{wm}) from a uniform distribution in the interval $[-2s_m, +2s_m]$ for each of several different selection coefficients, s_m (**Figure 5**). In the case of $s_m = 0$, we regain the typical game plot, representing the different classical games and their outcomes. Without game interactions, the evolutionary simulation will result purely in the fittest genotype. In the case of $s_m = 0$ the population is a heterogeneous mixture.

The equilibrium distribution of a system with known monoculture fitnesses and no game interactions is well defined and understood in population genetics. As a result, the equilibrium distributions/evolutionary outcomes measured in experiments are often assumed to be the result of such pure genetic fitness differences. This assumption does not account for potential game interactions between populations. As seen in **Figure 1**, the survival of the fittest (under which the 'fittest' genotype prevails), can result in multiple populations co-occurring and becomes population and interaction dependent.

Simulating modification, maintenance, masking and mimicry

In order to validate the theoretical restrictions (on α_{wm} and α_{mw}) that would result in the modification, masking or maintenance discussed above we simulated the dynamics of an evolving population with and without the interaction terms (**Figure 5**). We demonstrate several examples of added interaction terms and the modified evolutionary dynamics on a single allele landscape. We simulate the evolution of an initial population that is entirely made up of the 0 genotype (wildtype) on both a flat and peaked landscape, the model consists of a population of $1,000$ individuals undergoing mutation and selection. Without initial selection differences, neutral evolution occurs on the flat genetic landscape, whilst specific selection of the mutant is recapitulated with

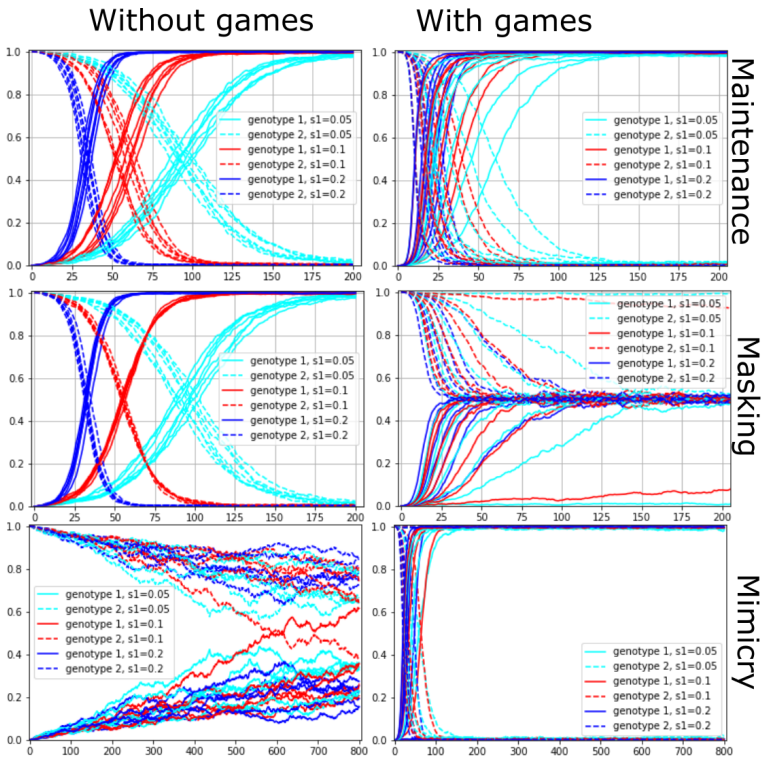

Figure 5. Maintenance, Masking and Mimicry Simulations with and without games are carried out for the one genotype case. An initial wildtype (0) population is evolved on a flat two genotype landscape and on the same genetic landscape but with added ecological epistasis. The evolution of the new system with game interactions can be the same or different depending on the game. Even in the maintenance case we observe alterations in the trajectory over time.

interaction terms added. Conversely, a mixed population is observed when game interactions of appropriate strengths are added that mask the underlying selective landscape.

Discussion

It is already understood in experimental biology that the presence of game interactions in addition to the underlying genotypic fitness can modify the evolutionary outcome of an evolving population. The extent to which this can happen, its parameterization and the incorporation of this effect into treatment plans is much less understood. One reason is that in traditional game theory, the notion of the game does not exist when there exists only one type of strategy present. This is in stark contrast to biology, where the survival and proliferation of an individual are of critical interest. In fact, in population dynamics, biology and in laboratory experiments where cell lines are isolated and grown, monoculture fitnesses of cells are typically the most well studied property. By reframing the game matrix such that the game interactions and monoculture fitnesses are separate terms, we come to a form that can both be interpreted within Fokker-Planck formalisms but also better understood from the perspective of evolutionary population dynamics.

We derive a general expression for the equilibrium distribution of a population obeying the Fokker-Planck equation with added game interactions. We reveal using mathematics and simulations the potential impact of game interactions to completely alter evolutionary dynamics. We find the critical boundaries at which these game dynamics either maintain a population at the originally fitter genotype (maintain), move a population from the fixation of the fitter genotype in monoculture to the fixation of the other (mimic), or even promote the heterogeneity of a population by levelling the playing field (masking). This result means that the measured outcome of any mixed population, such as tumor cells, must be interpreted with caution. Critically, the (observed) fitness of the cells in question may be significantly altered by cell-cell interactions. As such it may be that growth dynamics in mixed populations may bear little resemblance to monoculture growth rates and that initial experimental or metastatic seeding ratios may have strong impacts on resultant dynamics. Without specific techniques designed to robustly assay frequency-dependent game interactions[6,35], the magnitude of cell-extrinsic interactions cannot be quantified. These effects have widespread potential ramifications, for example when assaying chemotherapeutic drugs in isolated cell lines, when

developing cancer cell lines *ex vivo*, and when interpreting evidence for neutral evolution in tumors[36–40].

Whilst the first part of our work focused on the equilibrium distribution at long time periods, we also observed that systems with games present often have altered forms of evolutionary dynamics over time. This has been noted in previous work[14], where Kaznatcheev demonstrated that evolution is in some cases accelerated by game interactions. We observed that the magnitude of stochastic fluctuations in population size appeared distinct between game and non-game cases. In order to explore this property further, we suggest future work has the potential to derive explicit signatures of game dynamics encapsulated in the shape of the equilibrium solutions. Identifying these factors may be critical in interpreting the existence and strength of cell-cell interactions in experimental populations. Decomposition of the payoff matrix provides a biologically meaningful formulation of the payoff matrix and the ability to independently modify the cell-intrinsic and cell-extrinsic contributions to growth rate. In addition to supplying a new modelling paradigm and analytical solution in the case of added noise, this formalism provides an ideal starting point for the integration of existing pharmacokinetic understanding into game theoretical models. This model framework also more readily permits the integrated modelling of evolution, treatment and control in the presence of experimentally motivated drug and micro-environmental-dependent cell-cell interactions[35].

Methods

Mathematics

Deterministic formulation The payoff matrix P in a 2 strategy differential game and its subsequent decomposed form can be written as follows;

$$P = \begin{pmatrix} a & b \\ c & d \end{pmatrix} = \begin{pmatrix} g_w & g_w + \beta_{wm} \\ g_m + \beta_{mw} & g_m \end{pmatrix} = g_w \begin{pmatrix} 1 & 1 + \alpha_{mw} \\ 1 + s_m + \alpha_{wm} & 1 + s_m \end{pmatrix} \tag{11}$$

where s_m is the selective advantage of the mutant cell, $a = g_w$ is the growth rate of the wildtype cell, $d = g_m = (1 + s_m)g_w$ represents the growth rate of the mutant cell , β_{ij} is the modifying effect on growth rate of population j on population i and α_{ij} is the growth-normalised interaction effect of population j on population i. The payoff matrix without interaction A_0, can therefore be written as follows;

$$A_0 = \begin{pmatrix} g_w & g_w \\ g_m & g_m \end{pmatrix},$$

The replicator equation which describes the evolutionary dynamics of replicators with M possible strategies in general has the following form;

$$\dot{x} = x_i((Px)_i - x^T Px), \tag{12}$$

where x_i is the i^{th} population fraction and $i \in [1, M]$.

Stochastic formulation One method for describing the temporal evolution of a system with drift and diffusion is the Fokker-Planck equation as follows;

$$\frac{\partial \rho(x,t)}{\partial t} = -\frac{\partial}{\partial x}\Big[v(x)\rho(x,t)\Big] + \frac{\partial^2}{\partial x^2}\Big[D(x)\rho(x,t)\Big] \tag{13}$$

where $\rho(x,t)$ is the probability, at time t for a sub-population to make up fraction x of the population, $v(x)$ is the drift coefficient, and $D(x)$ is the diffusion coefficient.
The Kimura solution[34] to the Fokker-Planck equation is a Gaussian with the following potential;

$$\Phi = -2N(\mu \log(x) + \mu \log(1-x) + s_m x + \log(x(1-x))). \tag{14}$$

where x is the wildtype proportion, $1-x$ the mutant proportion, μ the mutation rate, s_m the selective advantage of the mutant and N the population size. We alter the selection to become frequency dependent by introducing a generic selection function, $f(x)$ with the aim to find a possible form;

$$\Phi = -2N(\mu \log(x) + \mu \log(1-x) + f(x) + \log(x(1-x))). \tag{15}$$

Maintenance The general dependence of the relationship between interaction coefficients in the case where the effective selection is maintained after interactions are added ($s_m = \sigma_m$);

$$\alpha_{wm} = \frac{(\alpha_{mw} + \sigma_m)(2\mu + \sigma_m - \sqrt{4\mu^2 + \sigma_m^2})}{4\mu^2 + (1+\sigma_m)(\sigma_m + \sqrt{4\mu^2+\sigma_m^2}) - 2\mu(1+\sigma_m+\sqrt{4\mu^2+\sigma_m^2})}. \tag{16}$$

Mimicking The general dependence of the relationship between interaction coefficients in the case where the effective selection is created by interactions alone ($s_m = 0$);

$$\alpha_{wm} = \frac{\alpha_{mw}(2\mu + \sigma_m - \sqrt{4\mu^2 + \sigma_m^2}) - \sigma_m(-2\mu + \sigma_m + \sqrt{4\mu^2+\sigma_m^2})}{4\mu^2 + (1+\sigma_m)(\sigma_m + \sqrt{4\mu^2+\sigma_m^2}) - 2\mu(1+\sigma_m+\sqrt{4\mu^2+s_m^2})} \tag{17}$$

Masking The general dependence of the relationship between interaction coefficients in the case where the effective selection is neutralised by interactions ($\sigma_m = 0$);

Simulations

We used our Python based ABM/CA model to observe the evolutionary trajectories before and after the addition of games, in particular asking whether our simulation results are consistent with game interactions designed to mask, mimic or maintain an evolutionary outcome.

The simulation involved a constant population of size N comprised of two species, denoted wildtype ('0') and mutant ('1'), undergoing mutation (rate μ) and selection at each generation. The sampling frequency of each population was based upon frequency-dependent fitness calculated at each generation. The constant population size N, mutation rate μ, mutant advantage s_m were all predetermined and a set of random payoff matrices were used. Simulated populations evolved from initial fraction x for 1000 generations, at which point the population fraction from each simulation was extracted.

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Code and data availability

All of the Mathematica and Python scripts in this project can be found on github at https://github.com/sbarkerclarke-phd/GamesModifyEvolution. All processed data needed for reproduction of the results of the paper are available in the same repository. All raw data files were published on GEA (accession number GSE98787).

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Supplemental

0.1 Derivations

We describe a system of interacting wild-type cells and mutant cells in the framework of the Wright-Fisher model. Each genotype follows stochastic replicator dynamics (a form of geometric Brownian motion) with fixed carrying capacity. In section 0.1.1, we write our model in a reduced form such that we use the minimum number of parameters to describe our system. In what follows, we describe the states of the model, how fitnesses and selection coefficients are calculated, and how interactions modify these fitnesses and selection coefficients.

In section 0.1.2 we derive the Fokker-Planck equation for a Wright-Fisher model of a haploid population, including the effects of mutation, and interaction dependent selection.

0.1.1 Interaction Selection Coefficient

The state of our system is given by a vector of frequencies:

$$\begin{aligned}\vec{x} &= \begin{pmatrix} x_w \\ x_m \end{pmatrix} \\ &= \begin{pmatrix} x_w \\ 1-x_w \end{pmatrix} \\ &= \begin{pmatrix} x \\ 1-x \end{pmatrix}\end{aligned} \tag{18}$$

The second component is justified by the requirement that the components of the state vector must sum to 1. The fitnesses of the wild-type and mutant without interactions are given by f_w and f_m respectively. Additionally, we have made the notational simplification $x^w \to x$. We define a selection coefficient (s_m) which reflects the relative fitnesses between the mutant and the wild-type without interactions:

$$s_m = \frac{f_m}{f_w} - 1 \tag{19}$$

In our model we allow for interactions, whose strengths are modulated by the parameters α_{wm} and α_{mw}. Addition of interactions modifies the selection coefficient by modifying the fitnesses of each genotype. These fitnesses may be calculated if one knows the form of a payoff matrix as well as the state vector. In the language of game theory, the genotypes are "strategies", and the payoff matrix defines a "game". Here we assume the genotypes are playing a symmetric game. In this case, the distinction between a genotype being "player 1" or "player 2" does not matter. The payoff matrix may be written (in a reduced form) as follows:

$$\begin{aligned}P &= \begin{pmatrix} P_{ww} & P_{wm} \\ P_{mw} & P_{mm} \end{pmatrix} \\ &= P_{ww} \begin{pmatrix} 1 & P_{wm}/P_{ww} \\ P_{mw}/P_{ww} & P_{mm}/P_{ww} \end{pmatrix} \\ &= P_{ww} \begin{pmatrix} 1 & 1+\alpha_{wm} \\ 1+s_m+\alpha_{mw} & 1+s_m \end{pmatrix} \\ &\overset{P_{ww}\to 1}{=} \begin{pmatrix} 1 & 1+\alpha_{wm} \\ 1+s_m+\alpha_{mw} & 1+s_m \end{pmatrix}\end{aligned} \tag{20}$$

Here P_{ww} is a scaling factor, which we set to 1. In the presence of interactions our fitnesses are now ϕ_w and ϕ_m, and the selection coefficient (s_m) is now:

$$\sigma_m(\vec{x}) = \frac{\phi_m}{\phi_w} - 1 \tag{21}$$

The interaction fitnesses are given by the expected payoff:

$$\begin{aligned}\vec{\phi} &= P\vec{x} \\ &= \begin{pmatrix} 1 & 1+\alpha_{wm} \\ 1+s_m+\alpha_{mw} & 1+s_m \end{pmatrix} \begin{pmatrix} x \\ 1-x \end{pmatrix} \\ &= \begin{pmatrix} 1+\alpha_{wm}-\alpha_{wm}x \\ 1+s_m+\alpha_{mw}x \end{pmatrix}\end{aligned} \tag{22}$$

Plugging the interaction fitnesses, we now have the interaction selection coefficient:

$$\begin{aligned}\sigma_m(\vec{x}) &= \frac{\phi_m}{\phi_w} - 1 \\ &= \frac{1+s_m+\alpha_{mw}x}{1+\alpha_{wm}-\alpha_{wm}x} - 1 \\ &= \frac{s_m-\alpha_{wm}+(\alpha_{wm}+\alpha_{mw})x}{1+\alpha_{wm}-\alpha_{wm}x}\end{aligned} \tag{23}$$

Plugging in $\alpha_{wm} = \alpha_{mw} = 0$ returns the selection coefficient without games, *i.e.* $\sigma_m(\vec{x}) = s_m$. Additionally $\sigma_w(\vec{x}) = s_w = 0$ in both the interacting case, and the non-interacting case.

0.1.2 Fokker-Planck Equation for a Modified Wright Fisher Model

The standard Wright-Fisher Model describes the change in frequency of alleles in a population. Parents are chosen randomly in a uniform way with replacements. These parents have offspring, which form the next generation of parents. In a parental population of N organisms and G possible alleles, we define the frequency of each allele (A_i) as x_t^i. The full set of frequencies is a state vector given by $\vec{x}_t = (x_t^1, \ldots, x_t^G)$. Addition of selection and mutation modifies the probability that a certain allele is chosen[34]. Instead of choosing parents based on the frequency vector $\vec{x}_t$, we choose parents based on the frequency vector $\vec{\psi}(\vec{x}_t)$. For the population described in section 0.1.1, we assume a constant mutation rate (μ). In order to obtain the Fokker-Planck equation, we must consider infinitesimally small time increments ($\delta t \ll 1$) and how, at these time scales, $\vec{\psi}(\vec{x}_t)$ drifts. The specific equations for $\psi^i(\vec{x}_t)$ are similar to the standard modification to $\vec{x}_t$, except we include an interaction-selection coefficient σ_m instead of s_m:

$$\begin{aligned}\psi^i(\vec{x}_t) &\approx x_t^i + ((1-2x_t^i)\mu + x_t^i(\sigma_i(\vec{x}_t) - \sigma_m(\vec{x}_t)(1-x_t)))\delta t \\ &= x_t^i + v_i(\vec{x}_t)\delta t\end{aligned} \tag{24}$$

where $v_i(\vec{x}_t)$ is a drift coefficient to be used in the Fokker-Planck equation. Here, we are interested in the time dependence over multiple generations. Instead of simply writing $\vec{x}$, we now have $\vec{x}_t$.

Since $x_t^m = 1 - x_t^w = 1 - x_t$, we may directly consider a 1-dimensional system. In this case, we consider:

$$\begin{aligned}\psi(x_t) &\approx x_t + ((1-2x_t)\mu + x_t(\sigma_w(x_t) - \sigma_m(x_t)(1-x_t)))\delta t \\ &= x_t + ((1-2x_t)\mu - \sigma_m(x_t)x_t(1-x_t))\delta t \\ &= x_t + v(x_t)\delta t\end{aligned} \tag{25}$$

where $v(x_t)$ is a drift coefficient to be used in the 1-dimensional Fokker-Planck equation. The drift coefficient can alternatively be written as:

$$\begin{aligned}v(x_t) &= (1-2x_t)\mu - \sigma_m(x_t)x_t(1-x_t) \\ &= (1-2x_t)\mu - \sigma_m(x_t)g(x_t)\end{aligned} \tag{26}$$

Here, $g(x_t) = x_t(1-x_t)$. We define the diffusion coefficient as:

$$D(x_t) = \frac{g(x_t)}{2N} \tag{27}$$

Taking the continuum limit in space and time, we have the following drift and diffusion coefficients:

$$\begin{aligned} v(x) &= (1-2x)\mu - \sigma_m(x)g(x) \\ D(x) &= \frac{g(x)}{2N} \end{aligned} \tag{28}$$

And the 1-dimensional Fokker-Planck equation is given by:

$$\frac{\partial \rho(x,t)}{\partial t} = -\frac{\partial}{\partial x}\Big[v(x)\rho(x,t)\Big] + \frac{\partial^2}{\partial x^2}\Big[D(x)\rho(x,t)\Big] \tag{29}$$

We are interested in the equilibrium solution $\rho^{eq}(x)$, which is the value of $\rho(x,t)$ at which the time derivative is zero. We now make an Ansatz that $v(x) = -D(x)\frac{\partial \Phi(x)}{\partial x}$ for some function $\Phi(x)$. In this case $\rho^{eq}(x) = R\exp(-U(x))$, which is the form of a Boltzmann-like distribution. Here $U(x) = \Phi(x) + \ln(D(x))$ and R is a normalization constant. We solve for $\Phi(x)$ in the following way:

$$\begin{aligned} v(x) &= -D(x)\frac{\partial \Phi(x)}{\partial x} \\ \frac{\partial \Phi(x)}{\partial x} &= -\frac{v(x)}{D(x)} \\ \int \frac{\partial \Phi(x)}{\partial x}dx &= -\int \frac{v(x)}{D(x)}dx \\ \Phi(x) &= -\int \frac{v(x)}{D(x)}dx \end{aligned} \tag{30}$$

Here, we do not need to worry about the constant of integration since it can be absorbed into the normalization constant R. Plugging in the expressions for $v(x)$ and $D(x)$, we have:

$$\begin{aligned} \Phi(x) &= -\int \frac{v(x)}{D(x)}dx \\ &= -\int \frac{(1-2x)\mu - \sigma_m(x)g(x)}{g(x)/(2N)}dx \\ &= -2N\mu\int \frac{1-2x}{g(x)}dx + 2N\int \sigma_m(x)dx \\ &= -2N\mu\int \frac{1-2x}{x(1-x)}dx + 2N\int \frac{s_m - \alpha_{wm} + (\alpha_{wm}+\alpha_{mw})x}{1+\alpha_{wm}-\alpha_{wm}x}dx \\ &= -2N\left[\mu\ln(x(1-x)) + \frac{(s_m-\alpha_{wm})\alpha_{wm} + (\alpha_{wm}+\alpha_{mw})(1+\alpha_{wm})}{\alpha_{wm}^2}\ln(1+\alpha_{wm}-\alpha_{wm}x) + \frac{\alpha_{wm}+\alpha_{mw}}{\alpha_{wm}}x\right] \end{aligned} \tag{31}$$

For the potential $(U(x))$ we have:

$$\begin{aligned} U(x) &= \Phi(x) + \ln(D(x)) \\ &= -2N\left[\mu\ln(x(1-x)) + \frac{(s_m-\alpha_{wm})\alpha_{wm} + (\alpha_{wm}+\alpha_{mw})(1+\alpha_{wm})}{\alpha_{wm}^2}\ln(1+\alpha_{wm}-\alpha_{wm}x) + \frac{\alpha_{wm}+\alpha_{mw}}{\alpha_{wm}}x\right] \\ &+ \ln(x(1-x)/2N) \\ &= -2N\left[\left(\mu - \frac{1}{2N}\right)\ln(x(1-x)) \right. \\ &\left. + \frac{(s_m-\alpha_{wm})\alpha_{wm} + (\alpha_{wm}+\alpha_{mw})(1+\alpha_{wm})}{\alpha_{wm}^2}\ln(1+\alpha_{wm}-\alpha_{wm}x) + \frac{\alpha_{wm}+\alpha_{mw}}{\alpha_{wm}}x\right] - \ln(2N) \end{aligned}$$

We can move the factor of $-\ln(2N)$ into the normalization constant R to obtain:

$$\begin{aligned} U(x) &= -2N\left[\left(\mu - \frac{1}{2N}\right)\ln(x(1-x))\right. \\ &\quad \left. + \frac{(s_m - \alpha_{wm})\alpha_{wm} + (\alpha_{wm} + \alpha_{mw})(1+\alpha_{wm})}{\alpha_{wm}^2}\ln(1+\alpha_{wm} - \alpha_{wm}x) + \frac{\alpha_{wm}+\alpha_{mw}}{\alpha_{wm}}x\right] \\ &= (1-2N\mu)\ln(x(1-x)) + f(x) \end{aligned}$$

Where we have defined the "selection function" $f(x)$:

$$f(x) = -2N\left[\frac{(s_m - \alpha_{wm})\alpha_{wm} + (\alpha_{wm} + \alpha_{mw})(1+\alpha_{wm})}{\alpha_{wm}^2}\ln(1+\alpha_{wm} - \alpha_{wm}x) + \frac{\alpha_{wm}+\alpha_{mw}}{\alpha_{wm}}x\right] \tag{32}$$

For the equilibrium distribution we have:

$$\rho^{eq}(x) = R\exp(-U(x)) \tag{33}$$

and the normalization constant (R) can be found in the following way:

$$R = \left(\int_0^1 \exp(-U(x))dx\right)^{-1} \tag{34}$$

In this case, R is difficult to solve analytically for arbitrary coefficients s_m, μ, α_{wm}, and α_{mw}, but it can be computed numerically.