Computer simulations of cellular group selection reveal mechanism for sustaining cooperation

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HIGHLIGHTS

- We develop a Gillespie simulation platform for the study of group selection.
- We identify the importance of pure cooperator groups in selection for cooperation.
- We show that cooperation benefits from asymmetric division mechanisms.

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ABSTRACT

We present a computer simulation of group selection that is inspired by proto-cell division. Two types of replicating molecules, cooperators and defectors, reside inside membrane bound compartments. Cooperators pay a cost for other replicators in the cell to receive a benefit. Defectors pay no cost and distribute no benefits. The total population size fluctuates as a consequence of births and deaths of individual replicators. Replication requires activated substrates that are generated at a constant rate. Our model includes mutation between cooperators and defectors and selection on two levels: within proto-cells and between proto-cells. We find surprising similarities and differences between models with and without cell death. In both cases, a necessary requirement for group selection to favor some level of cooperation is the continuous formation of a minimum fraction of pure cooperator groups. Subsequently these groups become undermined by defectors, because of mutation and selection within cells. Cell division mechanisms which generate pure cooperator groups more efficiently are stronger promoters of cooperation. For example, division of a proto-cell into many daughter cells is more powerful in enhancing cooperation than division into two daughter cells. Our model differs from previous studies of group selection in that we explore a variety of different features and relax several restrictive assumptions that would be needed for analytic calculations.

1. Introduction

Emergence of cooperative elements, specifically in the domain of early life dynamics, has been a subject of interest for years (Niesert et al., 1981; Szathmáry and Demeter, 1987; Hogeweg and Takeuchi, 2003; Takeuchi and Hogeweg, 2009, 2012; Zintzaras et al., 2010; Ma and Hu, 2012; Vaidya et al., 2012; Bianconi et al., 2013; Ferreira and Campos, 2013). In this context, one can describe a population of cooperative molecules that benefit others in some way, and defector molecules that do not benefit others. A typical example is a population of RNA molecules of which some can catalyze the replication of other RNA molecules. While in its catalytically active state, an RNA may not be available as a template itself. Helping the other RNAs to replicate thus reduces the catalytic RNA's own replication chances. Assuming that the catalytic benefits of cooperative RNA molecules are additive, without population structure
such cooperators are exploited by defectors and will lose the competition (Hogeweg and Takeuchi, 2003).

Population structure, however, may help cooperation to flourish by increasing the interaction between similar molecules. Two frameworks have been suggested for this: (i) evolution on a 2-dimensional lattice (Boerlijst and Hogeweg, 1991; Szabó et al., 2002; Scheuring et al., 2003; Hogeweg and Takeuchi, 2003), and (ii) compartmentalization of the population (Niesert et al., 1981; Szathmány and Demeter, 1987; Zintzaras et al., 2010; Bianconi et al., 2013). Both of these approaches introduce population structure that favors cooperative elements (Nowak, 2006). Constraining the evolutionary dynamics to a dense 2D lattice may colocalize cooperative elements. This ensures more frequent mutual encounters and thus enhances their replication. Compartmentalization allows cooperative elements to group with each other. This also enhances their replication and limits the number of defectors that can exploit a cooperators. Compartmentalization models based on lipid membranes (proto-cells) have been popular; both from a theoretical (Niesert et al., 1981; Szathmány and Demeter, 1987; Takeuchi and Hogeweg, 2009; Zintzaras et al., 2010; Mavelli, 2012; Ma and Hu, 2012; Bianconi et al., 2013) and experimental (Oberholzer et al., 1995; Szostak et al., 2001; Hanczyc et al., 2003; Chen et al., 2005; Zhu and Szostak, 2009; Kurihara et al., 2011; Adamala and Szostak, 2013, 2013b) perspective. This is due to their resemblance to modern cells as well as the prebiotically plausible chemistry for the formation of fatty acids that can spontaneously aggregate into closed membranes (Szostak, 2011; Dzieciol and Mann, 2012).

Compartmentalization models generally appeal to group selection as the selective pressure working in favor of the cooperators. Various descriptions for group selection have been proposed and studied over the years (Wynne-Edwards, 1962; Wilson, 1975, 2004; Maynard Smith, 1964; Wade, 1977, 1978; Leigh, 1983; Nunney, 1985; Szathmány and Demeter, 1987; Goodnight and Stevens, 1997; Sober and Wilson, 1999; Boyd and Richerson, 2002; Kerr and Godfrey-Smith, 2002; Paulsson, 2002; Ono et al., 2003). Though it has been a controversial topic for decades (Okasha, 2006), multiple recent studies have described effective group selection dynamics (Traulsen and Nowak, 2006; Nowak, 2006; Fontanari et al., 2006; Killingback et al., 2006; Traulsen et al., 2008; Wilson and Wilson, 2008; van Veelen, 2009; van den Bergh and Cowdy, 2009; Scheuring, 2010; Wang et al., 2011; Luo, 2013; Simon et al., 2013; van Veelen et al., 2014). The specifics of group selection, including those applied to early life dynamics, differ from model to model. Some models define explicit fitness functions for groups that reproduce as a whole (Fontanari et al., 2006; van Veelen et al., 2014). Other models have group level events, such as fission, without an explicit fitness assigned to the groups (Traulsen and Nowak, 2006; Bianconi et al., 2013; Simon et al., 2013). As in the models of the second group we use the definition of group selection as the effect of introducing group level events to a well-mixed population on the fitness of its individuals (Nowak, 2006; Simon et al., 2013). In existing group selection models the parameter domain is often simplified to make the model mathematically tractable. One common assumption is allowing only fixed group size or fixed population size (Fontanari et al., 2006; Traulsen and Nowak, 2006; Traulsen et al., 2008; Hauert and Imhof, 2012; Bianconi et al., 2013; van Veelen et al., 2014). These approaches have provided tremendous insight into the possibilities of group selection. Nonetheless, due to the simplifications, a large variety of ways in which group selection can apply has not been studied. Also, the focus of these studies was generally dedicated to answering the question of when group selection occurs, and what phenomena are affected by it.

We model proto-cells containing RNA molecules, using simple chemical reactions and follow the dynamics using the stochastic simulation approach (SSA) by Gillespie. This SSA approach provides an exact stochastic simulation of coupled chemical reactions (Gillespie, 1977; Gillespie et al., 2013). Although chemical kinetics is most often described using ordinary differential equations, this simulation approach is widely used as well for a number of reasons. First, a discrete-stochastic approach can be essential in the analysis of cellular systems. As the number of specific reactants inside the cell can be small, the behavior may deviate largely from model predictions obtained from the deterministic equations (Biancalani et al., 2012; RamaSwamy et al., 2012). However, for large numbers of molecules the results of the stochastic simulations typically only oscillate around the deterministic solution, where the amplitude of these oscillations decreases as the number of molecules simultaneously grows (van Kampen, 1992). This gives a second, very different, motivation for using the discrete-stochastic approach as recently demonstrated in the context of supramolecular copolymerization. Copolymerization of two different types of molecules results in an exploding number of possible aggregates, each with their own differential equation, making it very difficult to describe the system as the maximum aggregate size grows. However, stochastic simulations remain possible when one only follows the aggregates actually present (Markvoort et al., 2011).

Here we apply this simulation approach to follow proto-cells with widely varying composition of cooperators and defectors to study group selection. All RNA molecules (individuals) interact and replicate within their own proto-cell (group), which divides upon reaching a certain size. Using this simple, yet flexible, approach, which has similarities with the approach by Simon et al. (2013), we investigate the underlying mechanisms of group selection and shift the central focus of investigation into how and why group selection is effective. We can study large populations over sufficiently long time scales to observe both the dynamics and the steady state behavior. The flexibility of our approach provides the possibility of studying varying group and population size and composition, which is generally not suitable for analytical models. Thanks to this constraint relaxation, we make novel empirical observations on how the introduction of groups can affect individual fitness. We utilize our platform to simulate group selection over a range of parameters including mutation rates, group sizes, and division mechanisms. We believe that our approach reveals interesting properties of the dynamics of group selection that have not been observed previously, and could be helpful in describing events such as those occurring in an early life scenario.

2. Model and simulation approach

We consider a population of proto-cells containing two types of RNA molecules, cooperators C and defectors D. Cooperators have a catalytic function that helps other molecules in the cell to replicate. Defectors lack this catalytic function. We study the dynamics of such a population where (i) the molecules are chemically copied giving rise to a new molecule in the same cell, (ii) molecules degrade with rate θ, (iii) cells die with rate φ, and (iv) cells divide when they reach a maximum capacity m.

Replication of an RNA molecule is modeled as a bimolecular chemical reaction

\[
X + A \xrightarrow{k_+} X + Y
\]

(1)

Here \(k_+\) is the rate constant of the reaction and \(X\) is the RNA template molecule that is copied. This can either be a cooperators C or a defector D. The \(A\) are activated nucleotides that are the building blocks of the new RNA molecule \(Y\). This new molecule \(Y\) is not necessarily an exact copy of template \(X\). Mutations occur with probability \(u\). Thus with probability \(1 - u\) the copy of a cooperators will be another cooperators, while with probability \(u\)
it will be a defector. Symmetrically, the copy of a defector will be a cooperator with probability \( u \) and a defector with probability \( 1 - u \).

The catalytic effect of the cooperator molecules is incorporated by making the rate constants \( k_i \) a function of the contents of the cell the reaction occurs in. For this we use the following game theoretic approach where the rate constants \( k_i \) resemble the individual fitness of the molecules

\[
k_x(i,j) = \exp(\beta F_X(i,j))
\]

where \( \beta \) denotes the intensity of selection (\( 0 \leq \beta \leq 1 \)) and \( F_X(i,j) \) the payoff of a molecule of type \( X \) in a cell with \( i \) cooperators and \( j \) defectors. The payoff of a cooperator in this cell is given by (Nowak, 2006b)

\[
F_C(i,j) = \frac{R(i-1)+S_j}{i+j-1}
\]

In this formula \( R \) is the payoff for a cooperator when it interacts with another cooperator and \( S \) its payoff when it interacts with a defector. Analogously, the payoff of a defector in the same cell is defined by

\[
F_D(i,j) = \frac{Ti+P(j-1)}{i+j-1}
\]

where \( T \) is the payoff for a defector when it interacts with a cooperator and \( P \) its payoff when it interacts with another defector. Although the approach can be used for any values of \( R, S, T \) and \( P \), we focus on the interactions between cooperators and defectors that obey a Prisoner’s Dilemma payoff scheme. Cooperators pay a cost \( c \), providing their interaction partners a benefit \( b \), where \( b > c > 0 \). Defectors do not pay any cost and do not provide any benefit, but can benefit from cooperators (in mixed cells). In such a payoff scheme \( R, S, T \) and \( P \) are specified as in the following payoff matrix:

\[
\begin{pmatrix}
C & R = b - c & D \\
R = b - c & S = -c & D \\
T = b & P = 0 & D
\end{pmatrix}
\]

The growth of the system is controlled using the constant degradation rates for individual molecules \( \theta \) and complete cells \( \varphi \) in combination with the competition for the activated nucleotides required for replication. As the replication is described by the bimolecular reaction equation (1), the replication rate depends on the product of the rate constant and the concentration of activated monomers \( (A) \) available. The latter is controlled by the production (inflow) of activated monomers with constant rate \( \alpha \) and the degradation (outflow) of activated monomers present with rate constant \( \gamma \).

Cells divide upon reaching a maximum number of molecules \( m \). The daughter cells together inherit the entire contents of their parent. As a basic model, we consider division into two daughters with a combinatorial assignment of individuals (Bianconi et al., 2013). In this model, the composition of the daughter cells is determined by random sampling from all possible partitions into two subsets of the set of individuals in the parent cell. At the end, we compare the effects of this division mechanism with several other scenarios suggested in recent literature (Traulsen and Nowak, 2006; Zhu and Szostak, 2009; Simon et al., 2013).

The simulations are schematically illustrated in Fig. 1. The dynamics is simulated by calculating the propensities of all possible events in the entire system and then randomly selecting one of these events proportional to these propensities. Continuously repeating these steps, the simulations provide temporal trajectories of the number of cells and the number of cooperators and defectors in each of the cells. Both the number of molecules and the number of cells varies throughout the trajectory. Averaging over multiple simulations can yield estimates of any

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**Fig. 1.** Schematic representation of the stochastic simulations of cellular group selection. (a) The propensities of all possible reactions are calculated and one reaction is selected randomly proportional to these propensities. Here, for clarity, a system of only two proto-cells is shown, with a total of four cooperators (green) and one defector (red), together with all possible reactions: the inflow of activated nucleotides \( (p_0) \); the outflow of activated nucleotides \( (p_1) \); the removal of one defector \( (p_2) \) or one cooperator \( (p_3, p_4) \) from any of the cells; the creation of a new cooperator \( (p_6, p_9) \) or defector \( (p_5, p_7) \) of any of the cells; and the removal of one of the cells \( (p_8, p_{10}) \). By drawing a random number \( r \) between 0 and the sum of all propensities \( S \), in this example reaction 5 is selected. (b) Starting from an initial configuration, here consisting of four proto-cells, one reaction is selected randomly (as illustrated in part a) and performed and time advances by a random number drawn from an exponential distribution with a parameter equal to the sum of all reaction propensities \( J \). Next, the propensities of all possible reactions for the new configuration are calculated, etc. Moreover, once the contents of a cell reaches a certain threshold \( \theta \), in this case \( \theta = 5 \), the cell divides. In the simulations both the total number of molecules and the number of groups thus varies and there are no fixed time steps, nor discrete generations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
expected value that is computable from the chemical master equation (Gillespie et al., 2013). Moreover, the larger the number of molecules, the closer the temporal trajectories approximate the continuous-deterministic time-evolution of the reaction rate equations, which are the ordinary differential equations describing the same reactions. We start from an arbitrary initial configuration, simulate sufficiently long for the system to reach its steady-state, and continue the simulation to collect averages on this steady-state of the system. Further details on the simulations are given in Appendix A.

3. Results and discussion

3.1. Defectors thrive in the well-mixed system

First, we consider a single cell that never divides nor dies. Individual molecules degrade at a constant rate \( \theta = 0.1 \) and the replication rate depends on the concentration of activated monomers as well as the number of cooperator and defector molecules present. Because molecules may interact with all other molecules in the whole system, this is commonly referred to as a well-mixed system. For a large population, with \( i > 1 \) and \( j > 1 \), the fraction of cooperators, \( x = i/(i+j) \), can be obtained (see Appendix B) from the root of the quadratic equation

\[
x^2 \left( \exp(-\beta c) - 1 \right) + x (1 + u - (1-u) \exp(-\beta c)) - u = 0
\]

For the limit of large population size, the fraction of cooperators can thus be solved analytically and is independent of the benefit \( b \). Apart from the mutation rate \( u \) it only depends on the product of the cost of cooperation \( c \) and the intensity of selection \( \beta \). Fig. 2a shows the resulting fraction of cooperators as a function of \( u \) for four different values of the intensity of selection \( \beta \). In this figure the lines denote the analytical solution of Eq. (6) while the points show the steady-state averages obtained from the stochastic simulations.

Fig. 2a clearly shows that for perfect reproduction \( (u = 0) \) the cooperators go extinct in a well-mixed system, whereas for random reproduction \( (u = 0.5) \) defectors and cooperators are equally abundant. For any intermediate mutation rate cooperators are consistently less abundant than defectors. In case of weak selection \( (\beta = 1) \) the fraction of cooperators remains close to 50% for most mutation rates. In the remainder we therefore focus on strong selection, with \( \beta = 1 \). Intuitively, it can be argued that every cooperator is exploited by every defector in the population. Every cooperator pays a cost of helping a defector while receiving no benefit from that interaction. Hence, the cooperators are diluted further and further in the population and are only maintained due to mutations in the replication of defectors. Thus, as also observed previously, the well-mixed system always favors defection (Hogeweg and Takeuchi, 2003; Nowak, 2006). For mutation rates higher than 0.5, each defector is more likely to make a cooperator and vice versa. As this leads to paradoxical effects, we do not consider those results here.

3.2. Compartmentalization favors cooperation

Next, we consider the case where the molecules no longer occupy a single cell, but are divided over multiple cells instead. Individual molecules still degrade at the same constant rate \( \theta = 0.1 \) and replication still depends on the concentration of activated monomers available. However, the rate constant for replication is now solely affected by the other molecules within the same cell. Copies remain in the same cell as their templates. Cells divide when the number of molecules contained reaches a threshold \( m \) and disappear when they become empty.

The crosses in Fig. 2b show the fraction of cooperators in this group-structured population scenario for three different division thresholds \((m = 10, 25 \text{ and } 50)\). For comparison also the analytical solution for the well-mixed system is repeated. Three important observations can be made from this figure. For all thresholds: (i) the cooperators can dominate for sufficiently low mutation rates, (ii) the fraction of cooperators matches that of the well-mixed population for high mutation rates, and (iii) there exists a certain critical mutation rate, at the transition between these two regimes, where the fraction of cooperators is minimal. This critical mutation rate increases with decreasing division threshold \( m \).

In stark contrast to the well-mixed case, cooperators thus dominate in the group-structured population when the mutation rate is sufficiently low. The division over cells gives rise to an additional selective mechanism that favors cooperation. As the fate of the cooperators drastically changes upon introduction of group structure alone, we consider this group selection. Note, however, that the beneficial effects of cells only appear when the mutation rate drops below a certain threshold. For higher mutation rates, cooperators are mainly present by mutations of reproducing defectors, similar as in the well-mixed case, and their abundance decreases with decreasing mutation rate.

To study what influences the transition from the well-mixed-like behavior to domination of cooperators, we look into the distribution of the cell contents. In absence of mutations only pure cooperator cells are present. In case the division threshold \( m \) equals 25, these cells contain on average 9.7 molecules. Fig. 3a shows the distribution of
molecules over all possible cell types for six distinct mutation rates $u$. For $u=0.01$, the majority of cells still contain only cooperators. However, some mixed cells arise due to mutations. With increasing mutation rate mixed cells become the new majority, though pure cooperator cells remain present. For a given mutation rate mixed cells with a higher fraction of defectors tend to be smaller. This can be understood from the average fitness of molecules per cell, which typically decreases with increasing presence of defectors. With a constant death rate, cells in which molecules have an average fitness higher than the overall population will grow, whereas cells with molecules with lower average fitness are more likely to shrink. Up to a mutation rate of 0.07, the ratio of cooperators to defectors remains much larger than the corresponding ratio in the well-mixed system. Once the mutation rate passes its critical value, in this case between 0.07 and 0.08, the pure cooperator cells disappear and the mixed cell population shifts towards a much higher fraction of defectors. These high-fraction defector cells then also grow larger as they no longer have to compete with pure cooperator cells that have higher fitness, for the limited resources.

3.3. Cell death affects the cell composition but not the selection for cooperation

We also study the case where deaths occur at the cell level. With cell death rate $\phi = 0.1$, and individual molecule degradation rate $\theta = 0$, the rate at which molecules disappear (now as part of the cells) remains the same as before. Fig. 2b shows the fraction of cooperators as a function of the mutation rate for this cell death scenario. Interestingly, the curves for the individual degradation and the cell death scenarios match very closely. The choice of death mechanism thus appears unimportant to the steady-state frequency of cooperators in the population.

The relative abundance of molecules in different cell types for this cell death scenario is shown in Fig. 3b. Cells significantly smaller than $m/2$ are rare. This is because combinatorial division mainly results in daughter cells of roughly half their parent's size and cells cannot shrink in the absence of individual death. Without mutations only pure cooperator cells are present, similar to the individual death scenario. The average cell now contains 16.3 molecules.
molecules. For \( u = 0.01 \), also here the majority of cells still consists of only cooperators, with some mixed cells being formed due to mutations. Again, as the mutation rate increases to 0.06, mixed cells become the new majority while the cooperator to defector ratio in these mixed cells remains much larger than the ratio in the well-mixed system. The average fitness of individuals in a cell decreases when the defectors become more abundant. This slows down the growth rate of those cells, making them more likely to disappear than to reach division. Once the mutation rate passes its critical value, again between 0.07 and 0.08, also here the pure cooperator cells disappear and the mixed cell population shifts towards a higher fraction of defectors.

Mixed mechanisms where both individual molecules and complete cells can die are possible as well. Such simulations result in analogous curves for the fraction of cooperators as a function of the mutation rate as in Fig. 2b. The distributions of molecules over different types of cells look like combinations of the distributions in Fig. 3a and b.

### 3.4. Some pure cooperator cells are necessary for maintenance of cooperation

Although Fig. 2 showed that the fraction of cooperators is generally not affected by the death mechanism, Fig. 3 demonstrated that the distribution of those cooperators over cells is very different. Despite the obvious differences, for instance the mixed cells being much smaller for the individual degradation scenario than with cell deaths, an interesting similarity is noted. Namely, the critical mutation rate where the presence of cooperators starts to behave similarly to that of the well-mixed system in both cases coincides with the loss of a reservoir of pure cooperator cells. This is also illustrated in Fig. 4a where, for both the individual degradation and for the cell death scenarios, the fraction of cells only containing cooperators is shown as a function of the mutation rate. This suggests that the critical mutation rate for which the cell advantage comes into effect is related to whether pure cooperator cell are maintained in the system.

The role of the pure cooperator cells is further clarified in Fig. 4b and c. There we study the situation just below the mutation threshold, namely at \( u = 0.07 \). In Fig. 4b we compare the distribution of the fraction of cooperators in cells that reach division with that of all cells in the system. As expected, the cells with a high fraction of cooperators grow more quickly and are more likely to reach the maximum cell size. Further, we observe that even though pure cooperator cells are relatively rare at this mutation rate, they still constitute the most common type among cells reaching division. Fig. 4c shows that within mixed cells there is a constant dilution of cooperators by defectors. This occurs because in every mixed cell the replication rate of defectors is higher than that of cooperators. The figure also shows that pure cooperator daughters are only formed from cells that at their own birth consisted of a high fraction of cooperators as well.

For perfect replication, pure cooperator cells always remain pure while existing mixed cells continuously increase the fraction of defectors. As molecules in pure cooperator cells have a higher replication rate than those in pure defector cells, the division over cells thus eliminates defectors in the absence of mutation. This contrasts the well-mixed scenario, where the absence of mutation eliminates cooperators.

In presence of mutations, a pure cooperator cell may be infected by a mutant defector. Once such a cell undergoes division, at least one of its daughters will inherit a defector. In this ‘infected’ daughter, defectors are more likely to reproduce than cooperators. Hence the cell will dilute further and further. Along with the dilution, the replication rate of the molecules in the cell decreases. These cells grow slower and slower as they dilute, because they lose the competition for resources to the cells with more cooperators. This reduced growth rate makes them likely to disappear before they dilute completely. When the mutation rate becomes higher, multiple mutations may occur in a pure cell before it reaches division or the first mutation might occur early such that the defector is likely to replicate multiple times before division occurs. The more defectors in the cell the less likely it is that one of the daughter cells will again be pure. Once the mutation rate exceeds the critical boundary, and pure cooperator cells disappear, competition between mixed cells and pure cooperator cells is eliminated. Mixed cells that were not able to reach the division threshold before, now do. This further shifts the composition of mixed cells towards a higher fraction defector. Cooperators are not
completely eliminated due to mutations of defectors, just as in the well-mixed population.

In the individual death scenario the effect is similar though slightly different. Cells whose molecules average fitness is higher than the overall average fitness still grow. Thus, these cells divide eventually. In contrast, cells whose individuals have lower fitness shrink. Consequently most pure cells also contain between $m/2$ and $m$ molecules. Cells with mostly cooperators still grow as well, generally increasing their fraction of defectors. Once the fraction of defectors becomes too large, the cells start to shrink. Ultimately they become empty and disappear. Similar as in the cell death scenario, once no longer sufficient pure cooperator cells are present, mixed cells can increase in size due to the reduced competition for common resources. Mixed cells dilute again till the fraction of defectors reaches that of the well-mixed system. Another difference with the cell death scenario is that infected cells can become pure again by the degradation of a defector. However, because the fitness of a single defector in a cell with mostly cooperators is very high, the defector is much more likely to replicate than to degrade.

The necessity of pure cooperators to select for cooperation can also be reasoned from the fitness landscape. For cooperators to be most abundant, their average fitness should be larger than that of defectors. In a mixed cell the defectors always have a higher fitness than the cooperators. Consequently, the average fitness of cooperators can only be higher than that of defectors if a sufficient number of pure cells exist.

### 3.5. Occurrence of phase transition is robust to variations in cost and benefit of cooperation

Because the maintenance of pure cooperators cells proves essential to the success of cooperators, we next investigate the factors contributing to their presence. Fig. 5a and b depicts, for the cell death scenario, the fate of cooperators for a range of benefit $b$ and cost of cooperation $c$ values, respectively. This shows that the transition from well-mixed like behavior at high mutation rates to selection for cooperation at lower mutation rates is robust to variation of both the benefit and the cost of cooperation. It also shows that the critical mutation rate changes as benefit and cost are manipulated. Increasing benefit improves the mutation tolerance, up to a maximum point $u_{\text{max}}$. Higher $b$ values cease to affect the location of transition, though still increase the fraction of cooperators in the system for the same mutation rate. On the other hand, increasing cost $c$ lowers the tolerance down to $u_{\text{max}}$ but no further. After this point, growth of $c$ no longer affects the transition threshold, although it still decreases the fraction of cooperators in the system for the same mutation rate.

The results are consistent with our observations before. When the benefit $b$ decreases, the rate of growth for cooperative cells decreases as their relative fitness is proportionally smaller. As expected this makes pure cooperators cells harder to maintain and decreases the tolerated mutation rate. Understanding the effects of various cost values is more involved. Because pure cooperator cells contain no defectors and the payoff between cooperators is $b$ and $c$, the effect of increasing $c$ within a pure cooperator cell matches that of decreasing $b$. However, higher $c$ values do significantly increase the relative fitness of defectors. Consequently, a pure cooperator cell that has been infected by a defector becomes less likely to recover. Similar to decreasing $b$, this causes a drop in mutation tolerance. Once $c$ is so large that a defector is likely to yield multiple more defectors before the cell divides, it hardly matters how much larger $c$ is. Division will probably yield daughters that are both mixed. On the other hand, for very small values of $c$ the advantages of defectors start to vanish. Infected cells dilute only slowly and could still give rise to pure cooperator cells as they divide.

The maintenance of the pure cooperators cells will depend on one factor: does a pure cooperator cell make at least one other pure cooperator cell before it dies? We have already seen that due to dilution no other type of cell can maintain itself consistently. When $c$ is meaningfully large ($1 \leq b/c \leq 10$), appearance of a defector during replication has two effects. First, it has a high advantage relative to the other individuals in the cell and therefore it is more likely to replicate. Second, when the cell eventually divides at least one of the two daughter cells will contain a defector. To maintain, pure cooperators cells need to give birth to at least one pure daughter. With combinatorial division in place, the most likely scenario for such an event is when the cell has no more than one defector at division time. Since there are roughly $m/2$ replication events from when a cell is created until it divides, the expected number of defectors created should be less than one for the cell to have a pure daughter. Therefore, under the condition that no pure cells are recovered from cells that start as mixed, the upper bound on the mutation rate for which the transition occurs can be estimated by $u < 2/m$.

### 3.6. Asymmetric division boosts cooperation

The observation that pure cooperators cells should be maintained also lead us to investigate other division mechanisms that
might have different probabilities of recovering pure cooperator cells from mixed cells. In Fig. 6 the fraction cooperators is shown for six different division mechanisms, again for the cell death scenario with \( m = 25, b = 5 \) and \( c = 1 \). The first division mechanism (combinatorial) is the one used so far and is shown as the reference.

The second division mechanism division (equal) comprises division into two as equal as possible daughters (equal), division by randomly cutting linearly aligned but randomly ordered molecules (linear), division by randomly dividing molecules per type (linear per type), division into \( m \) single individual cells (many) and a mechanism where once size \( m \) is reached division only occurs with probability 0.001 and otherwise one random individual in the cell dies (sporadic). \( \beta = 1, m = 25 \) and cell death in all cases. The figure shows that, in accordance with the previous results, the more likely a division mechanism is to produce pure cooperator cells the easier it is for cooperation to flourish.

In the fifth division mechanism (many) a cell no longer divides in just two daughters, but into \( m \) cells containing a single molecule instead. This mechanism is based on the experimentally observed division of lipid vesicles into many daughters via a pearling transition (Zhu and Szostak, 2009). By definition all daughters are pure and each mixed cell thus results in at least one pure cooperator cell. The fraction cooperators with this mechanism are again higher than with any of the previous division mechanisms for all mutation rates larger than zero.

In the final division mechanism considered (sporadic) division no longer occurs automatically upon reaching size \( m \) (Traulsen and Nowak, 2006). Instead, it only occurs with probability \( q \), here taken as \( 10^{-3} \). Otherwise one random molecule in the cell degrades. Divisions are thus rare relative to replications. This allows a single defector in a cell of cooperators to take over the whole cell. Most cells will contain \( m - 1 \) molecules and consist either of only cooperators or only defectors. Pure cooperator cells can only maintain if none of the replications resulted in a mutation. Pure cooperator cells, and selection for cooperation, consequently only occur when mutation rates are very low. For higher mutation rates, the fraction of cooperators precisely follows the mutation rate.

All these results are in line with the earlier observation of the role of pure cooperators. Mechanisms that are more likely to produce pure cooperator cells result in higher selection for cooperation and less steep transitions.
growing group that creates offspring like itself is the pure cooperator group. The groups are helping the cooperators win, because they isolate them from defectors. Groups of mainly cooperators that contain even a single defector need to either eliminate it or face dilution. The longer the defector survives in a group, the more difficult it becomes to recover a purely cooperative group by either division or individual death of the defectors.

The division mechanism turns out to play a key role in whether group selection can be effective because division details highly influence the probability of yielding pure cooperator daughters. Mechanisms creating smaller groups or likely to isolate cooperators appear very advantageous to cooperators. The division mechanism could thus have played an important role in the maintenance of cooperative ribozymes in early life. This emphasizes the importance of studying possible alternative division mechanisms. In the first part of this study, we focused on the division into two where the contents of the daughter cells was randomly drawn from all possible divisions into two subset of the set of molecules in the dividing proto-cell. This seems a reasonable description of a set of molecules randomly diffusing inside a vesicle that divides into two. Such division has been observed both experimentally and using molecular dynamics simulations (Stano et al., 2006; Kurihara et al., 2011; Markvoort et al., 2010). But also other mechanisms might be well possible. For maintaining cooperation the pearling transition as observed by Zhu and Szostak (2009), which is a division into many, seems especially interesting. The more as it has also been observed in bacteria in which the cell division machinery was eliminated (Leaver et al., 2009). It would be interesting to see whether a, and if so what, specific division mechanism will be selected for in a competition between different division strategies. Intriguingly, the division mechanisms most favorable for the maintenance of cooperation are very asymmetric. In sharp contrast to the common division mechanisms of contemporary cells that tend to divide into two rather equal daughters.

Appendix A. Simulation details

In the simulations we follow the time evolution of molecules and their distribution over cells inside a volume V. We follow the common convention where rate constants for unimolecular reactions have unit s⁻¹ and those for bimolecular reactions M⁻¹ s⁻¹. Replication of a molecule is modeled as a bimolecular reaction; one cooperator (defector) molecule and one resource molecule yield two cooperator (defector) molecules. The rate constant for this reaction is given by the molecule’s fitness according to Eq. (2). The propensity of replication of one specific cooperator in a cell containing i cooperators and j defectors is thus given by \( f_C(i,j) = \frac{N_x}{N} \). Here \( N_x \) is the number of resource molecules (activated nucleotides), \( N \) Avogadro’s number and \( V \) the volume. Analogously, \( f_D(i,j) = \frac{N_x}{N} \) is the propensity of replication of one specific defector in that same cell. Degradation of individual molecules and of cells are modeled as unimolecular reactions. For each individual cooperator and defector the propensity to degrade is simply \( \beta \). For each cell the propensity to die is \( \phi \). Death of a cell implies loss of all cooperators and defectors in that cell. For each resource molecule the propensity to degrade/flow out is \( \gamma \). Production/flow of new resource molecules is modeled as a source term with rate constant \( \alpha \) with unit M s⁻¹. The propensity for a new resource molecule to appear is then \( \alpha N_x \). The simulations proceed by repeatedly calculating the propensities of all possible reactions and selecting one reaction randomly proportional to these propensities. Every reaction the time advances by a random number drawn from an exponential distribution with as parameter the sum of all reaction propensities.

For simulations of the well-mixed system, the initial configuration consists of a single cell with 10,000 cooperators and 10,000 defectors in a volume of 10⁻¹⁸ l. For the group structured simulations, an initial configuration with 1000 proto-cells, with half of them containing a single cooperator and the other half containing a single defector, is used in a volume of either 5 × 10⁻¹⁹ or 10⁻¹⁸. The system is then allowed 10⁷ reactions involving individuals and/or cells, thus without counting inflow and outflow of resource molecules, to reach steady-state. Then another 10⁷ reactions involving individuals and/or cells are followed to extract averages on the steady-state composition. In all simulations \( \alpha = 0.01 \) M s⁻¹, \( \gamma = 0.01 \) s⁻¹, and the degradation rate of individuals (either individual degradation rate \( \phi \) or via cell death rate \( \theta \)) is 0.1 s⁻¹. All these parameters can be scaled, but were chosen such that in the steady state of the well-mixed simulations in the order of 50,000 individuals were present, and in that of the group structured simulations between 1000 and 5000 cells containing between 25,000 and 60,000 individuals. In order to test that these systems are sufficiently large and the simulations sufficiently long, we calculated confidence intervals for some steady state averages using the batch means method (Law and Kelton, 1982). The half widths of the 95% confidence intervals for the fraction cooperators in Fig. 2 for instance all are (much) smaller than 0.015.

Appendix B. Well-mixed system

In the limit of large number of molecules \( i+j = N+1 \) the payoff for cooperators \( C \) as defined in Eq. (3) can be approximated by \( F_C(i,j) \approx R_x + S(1-x) \). Similarly, the payoff for defectors \( D \) as defined in Eq. (4) can be approximated by \( F_D(i,j) \approx T_x + P(1-x) \). In these equations \( x = i/N \) is the fraction cooperators. With the payoff matrix in Eq. (5) this reduces to \( F_C(i,j) \approx bx - c \) and \( F_D(i,j) \approx bx \). The fitness of a cooperator \( C \) is thus given by \( f_C(x) = \exp(bx - c) \) and that of a defector \( D \) by \( f_D(x) = \exp(bx) \). These are the rate constants for replication of the cooperators and defectors respectively. The replication rate of cooperators is thus proportional to

\[
if_C(i,j) \approx xN \exp(bx - c)
\]

and that of defectors

\[
if_D(i,j) \approx (1-x)N \exp(bx)
\]

Because replication occurs with accuracy \( (1-u) \), the birth rate of cooperators is thus

\[
r_C = (1-u)xN \exp(bx - c) + u(1-x)N \exp(bx)
\]

and that of defectors

\[
r_D = uN \exp(bx - c) + (1-u)(1-x)N \exp(bx)
\]

These rates can be simplified to

\[
r_C = N \exp(bx)((1-u)x \exp(-bc) + u(1-x))
\]

and

\[
r_D = N \exp(bx)(ux \exp(-bc) + u(1-x))
\]

If the degradation rate of individual molecules is independent of the type, in steady state the fraction of cooperators in the newly formed molecules should equal the fraction present in the system, i.e.,
This can be simplified to

\[ x = \frac{(1-u)x \exp(-\beta c) + (1-x)}{u x \exp(-\beta c) + (1-x)} \]

and further simplified to

\[ x = \frac{1 - u x + (1 - u) x \exp(-\beta c)}{x \exp(-\beta c) + 1 - x} \]

resulting in the quadratic equation

\[ x^2 (\exp(-\beta c) - 1) + (1 + u - (1 - u) x \exp(-\beta c) - u - 1 = 0 \]

The fraction cooperator (x) can thus be solved analytically from this as the root between 0 and 1, i.e.,

\[ x = \frac{-1 + (1 - u) x \exp(-\beta c) + (1 + u - (1 - u) x \exp(-\beta c) - u - 1)}{2(\exp(-\beta c) - 1)} \]

The fraction cooperator (x) is thus independent of the benefit. It only depends on the quality of reproduction, the cost of being a cooperator (c) and the intensity of selection (β).

References


