Unicellular propagules, cancer, and competition: Are unicellular propagules part of the optimal life-history strategy in the face of both cancer and competition?

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Abstract

Evolutionary transitions in individuality introduce new levels of selection and thus enable discordant selection, threatening the stability of the transition. Cancer is such a problem for multicellularity. So why have so many transitions to multicellularity persisted? One possibility is that the unicellular propagule maintains cooperation among cells by purging cheaters. The evolution of propagule size has been modeled previously, but in the absence of competition between individuals, which may often select for larger propagules. How does the nature of competition between individuals affect the optimal propagule size in the presence of cancer? Here we take a model of early multicellularity, add cancer, and simulate size-dependent competition on a lattice, which allows us to tune the strength of interspecific vs. intraspecific competition via dispersal. As expected, cancer favours strategies with unicellular propagules while size-dependent competition

favours strategies with few large propagules (binary fragmentation). How these opposing forces resolve depends on dispersal. Local dispersal, which intensifies intraspecific competition, favours binary fragmentation, which reduces intraspecific competition for space, with one unicellular propagule. Global dispersal instead favours multiple fission when cancer is common. Our results shed light on the evolution of multicellular life cycles and the prevalence of unicellular propagules across the tree of life.

Author summary

A multicellular organism is a group of cooperating cells. But wherever there is cooperation there is the temptation to cheat. Cancer cells are cheating cells. Having offspring that start as a single cell (the unicellular propagule) has been hypothesized as an adaptation to deal with these cheating cells by increasing kinship among cells in the organism and purging lineages of cancer cells. We model the evolution of offspring size but add competition between individuals, which may select against small unicellular offspring. We find that having some unicellular offspring is a successful strategy, but how many depends on the nature of competition.

Introduction

Selection acts on variation. Where there is heritable variation across individuals – be it at the level of the gene, cell, organism, or beyond – there is potential for evolution by

natural selection (Lewontin 1970). Selection acting at multiple levels simultaneously is known as multilevel selection (Szathmary and Maynard Smith 1995).

A multicellular organism is composed of cooperating cells. This life-history strategy has independently evolved multiple times (Grosberg and Strathmann 2007). One proposed advantage of multicellularity is the potential to be larger (Michod and Roze 2001), which may protect an individual from its environment, e.g., predators. Another possible advantage of multicellularity is division of labour. With multiple cells in an organism there is potential for cells to specialize, increasing efficiency.

Conflict between levels of selection can arise when the units at these different levels each have influence over a phenotype with opposing effects on fitness. For example, in the case of multicellularity, cell replication rate can have very different effects on the fitness of the cell and the fitness of the organism. When mutations overwrite the checkpoints that maintain cooperation between cells, selection on replication rate at the cell level can result in cancer. Cancer is a problem that all multicellular organisms must deal with and can be found across the tree of life (Atkipis et al. 2015). As much as cheating comes hand in hand with cooperation, cancer comes with multicellularity.

Most multicellular organisms begin as a unicellular propagule (Bell and Koufopanou 1991). Why is it that multicellular organisms go to the trouble of starting from a single cell, temporarily forgoing all the benefits that come with multicellularity? One hypothesis is that the unicellular propagule is an organism-level adaptation to maintain cooperation

among cells, i.e., to prevent cancer (Strathmann and Grosberg 2007; Strathmann and Grosberg 1998). The single-cell bottleneck reduces the risk of cancer in two ways. First, it purges lineages of cancer cells, preventing them from being vertically transmitted, by forcing selection on the cell and organism to align (because the organism is a single cell). Second, it increases kinship among cells within an organism, reducing the incentives and opportunities for cheating.

A number of mathematical models shed light on why the unicellular propagule is such a common strategy. Building on previous work (Otto and Orive 1995; Michod 1996; Michod 1997), Roze and Michod (2001) asked what number of cells in the propagule maximizes population growth rate when cancer cells continually arise from mutation and the fitness of an organism depends on both the proportion of its cells that are cancerous and its total number of cells (organism size). They found that the unicellular propagule, which reduces mutational load, maximizes population growth rate when the benefits of larger size are not too great. This model assumes non-overlapping generations, large adult sizes (since the cells in a propagule are sampled with replacement from the adult), and indeterminant growth.

A completely new approach to modeling the evolution of propagule size, more suitable for smaller, more primitive multicellular organisms, was introduced by Pichgin et al. (2017). Instead of choosing cells from an adult (with replacement) to create many propagules of the same size, they introduced the concept of fragmentation mode, the size of an adult and the way it splits apart to produce offspring. The partitions of the

number of cells in an adult determines its possible fragmentation modes. As an example, consider an adult made of four cells. This adult could fragment into four single cells (1+1+1+1); two groups of two cells (2+2); one group of two and two single cells (2+1+1); or one group of three and one single cell (3+1). These are four different fragmentation modes. Ignoring mutation, Pichgin et al. (2017) found that all fragmentation modes that maximize population growth rate create only two offspring (e.g., 2+2 or 3+1). This includes what they call the “unicellular propagule mode”, an adult having mode n+1 (e.g., 3+1), which is optimal in the absence of cancer when being large has outsized benefits. Given this is a model with overlapping generations, we can interpret the unicellular propagule mode as an adult continually producing unicellular offspring. It may therefore be, then, that the single-cell bottleneck does not primarily evolve to prevent cancer. Rather, it may simply maximize population growth rate. Adding frequency-dependent competition between organisms (Ress et al. 2022) does not affect the fact that all optimal modes have two offspring, but does allow for complications such as coexistence and bistability.

Mutations were added to the fragmentation framework by Gao et al. (2017), who used a payoff matrix to describe interactions between the two cell types. Similar to Pichugin et al. (2017), they found that the optimal strategies were the unicellular propagule mode and binary fission (e.g., 2+2), but also multiple fission into all single cells (e.g., 1+1+1+1). In particular, when a group composed of both cell types is disadvantageous at the group level (as is the case with cancer cells, which reduce the fitness of cooperating cells in the same organism) then multiple fission is optimal. A key

assumption of this model is that solitary cells do not play the game, removing the opportunity to purge cancer through unicellular offspring. These results may then be seen as support for the hypothesis that having unicellular offspring, while unable to purge cancer, maintains cooperation among cells by increasing kinship/decreasing genetic variation.

While the above models support the idea that the unicellular propagule can increase cooperation between cells in the face of cancer, for simplicity none of those models include interactions both between cells and between organisms. However, given that cancer is a multilevel selection problem, it may be informative to consider interactions between individuals at multiple levels. In particular, here we ask, are unicellular propagules part of the optimal life-history strategy in the face of cancer when there is also size-dependent competition between organisms? Previous models also ignore space. Because space interacts strongly with competition (Durrett and Levin 1994, Kerr et al 2002), tuning the strength of interspecific vs. intraspecific interactions, here we also ask how the unicellular propagule strategy fares with and without spatial structure.

In the presence of both cancer and size-dependent competition between organisms we expect that producing unicellular propagules will be part of the optimal strategy when the benefits of purging cancer outweigh the costs of having small offspring. For example, as the mutation rate to cancer increases we expect that strategies that produce unicellular propagules will outcompete those that don’t.

To test this hypothesis we simulate the fragmentation framework of Pichugin et al. (2017) on a lattice, where each vertex can contain at most one individual, with recurrent cancer mutation. We find that cancer, competition, and space together can select for many different life histories. In particular, increasing mutation rates favours the production of unicellular propagules, as expected. However, in the absence of spatial structure the optimal strategy at high mutation rates is multiple fission, not the unicellular propagule strategy. Adding spatial structure alters this conclusion, favouring the unicellular propagule strategy.

Materials and Methods

Below we give an overview of the simulation method. The full code, written in Python, is publicly available on GitHub (https://github.com/Sydneya1/Lattice-Model).

To start the simulation, N_0 single cells are randomly scattered on an M x M lattice and given a (pure) fragmentation mode (Pichugin et al. 2017). For an adult size L, the fragmentation modes are all partitions of L.

Each timestep starts with cell division. The rate at which a cooperator cell divides in a group with i cooperator cells is given by b_i. Below we will consider two cases of cell division rates (i.e., cell “birth rates”): 1) size independent, in which the rate of a cell dividing in a group is the same for groups of all sizes, and 2) size dependent, in which the rate increases linearly with the number of cooperators. Similar to Pichugin et al. (2017), these are given by

$$b_i = b + a\frac{i-1}{n-1}$$

where *n* is the largest adult size among the modes allowed in a given simulation. For size independent birth rates $a = 0$ and for size dependent birth rates $a = 1$. We assume that groups can increase by at most one cooperator cell per time step. This probability is given by

$$B_i = 1 - e^{-ib_i}$$

where *i* is the number of cooperator cells. When a cooperator cell divides, the new cell has probability *m* of mutating to a cancer cell. We assume no back mutation.

Groups with cancer cells also have some probability of growing by a cancer cell every iteration. The division rate of a cancer cell in a group of size *i* is

$$c_i = rb_i$$

where *r* >1 is the replicative advantage of cancer cells. The probability that a group with *i* cooperator cells and *j* cancer cells will increase by one cancer cell is given by

$$C_{ij} = 1 - e^{-jc_i}.$$

Note that in the simulations we assume cancer cell division happens before cooperator cell division. This means that if the group is one cell division short of reaching its max adult size and it grows by a cancer cell, then that precludes a cooperator cell from dividing. Additionally, groups with some cooperators and some cancer cells can grow by two cells per iteration, while groups with only one type of cell only grow by one cell per iteration. These limitations can be relaxed by making the time steps (and hence birth rates) small, such that multiple simultaneous cell divisions in a group of cells is small.

The next event in a time step is group death. There is a constant background mortality rate, *d*. This death rate is converted to a probability

$$D = 1 - e^{-d}.$$

Groups can also die with a probability *P* where *P* is the proportion of cancer cells in the group. Groups survive with probability (1-*D*)(1-*P*).

Finally, fragmentation, dispersal, and competition occur. Working through one group at a time, if it has reached its adult size it splits apart into offspring whose size and number are determined by its fragmentation mode. In this way, any cancer cells are randomly distributed among offspring. Each offspring is then randomly assigned a place on the lattice. Here there are two treatments, 1) global dispersal (no spatial structure) and 2) local dispersal (spatial structure). Under global dispersal each offspring has an equal chance of landing on any vertex on the lattice. Under local dispersal each offspring has an equal chance of dispersing to one of five neighboring vertices: on the parent vertex or one vertex above, below, right, or left of it.

In either case, if an offspring lands on a vertex that is already occupied, competition occurs. Under complete size-dependent competition the larger group will win the spot (if the vertex is occupied by a group of the same size then each has a 50% chance of winning the spot). Individuals that ‘lose’ the spot die. To isolate the effects of size dependence, we also model “random competition”, where every offspring has a 50% chance of winning an occupied vertex regardless of size. The gradient in-between these two extreme forms of competition can be explored by introducing a parameter *s* such that the probability of the larger individual winning a spot is (1+*s*)/2 , with *s* = 0

corresponding to random competition and s = 1 corresponding to complete size-dependent competition.

This process repeats for T timesteps. A visualization of this life cycle of cell division, group death, fragmentation, dispersal, and competition can be seen in Figure 1. An example of the lattice before and after the simulation, as well as a time series plot showing the dynamics of the population during the simulation can be seen in Figure 2. A description of the parameters used in the simulation are summarized in Table 2.

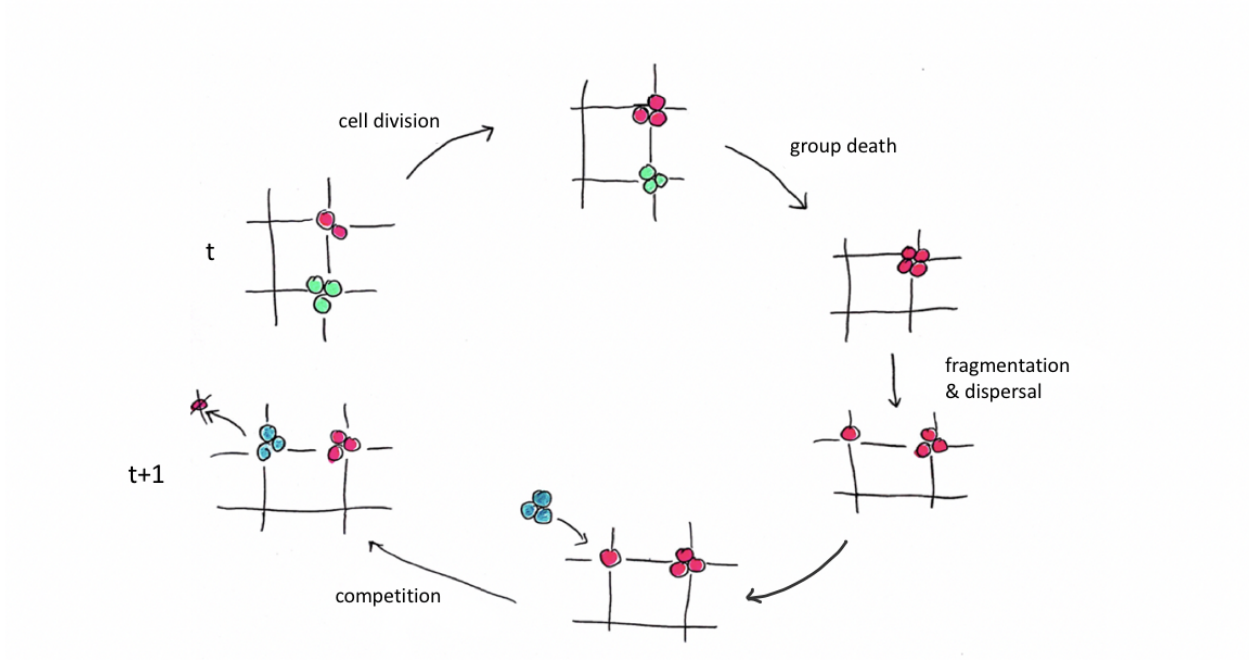

Figure 1. A sample of the lattice is shown to illustrate the simulated life cycle. The colours indicate the fragmentation modes, with pink being 3+1 (the modes of green and blue aren't indicated here as they don't fragment). We start on the left with two groups, pink of size 2 and green of size 3. During the cell division stage the pink group grows by one cell, while the green group does not grow. The next stage is group death. In this instance the green group dies. Next the pink group fragments since it has reached its

max size of L=4. Both pink offspring are assigned to empty vertices, but elsewhere on the lattice an offspring of size three (with the blue fragmentation mode) is assigned to the spot that the pink single cell currently occupies. In this case the blue offspring wins the spot and the pink offspring dies (as would happen with complete size-dependent competition). After fragmentation the time is updated and the life cycle begins again.

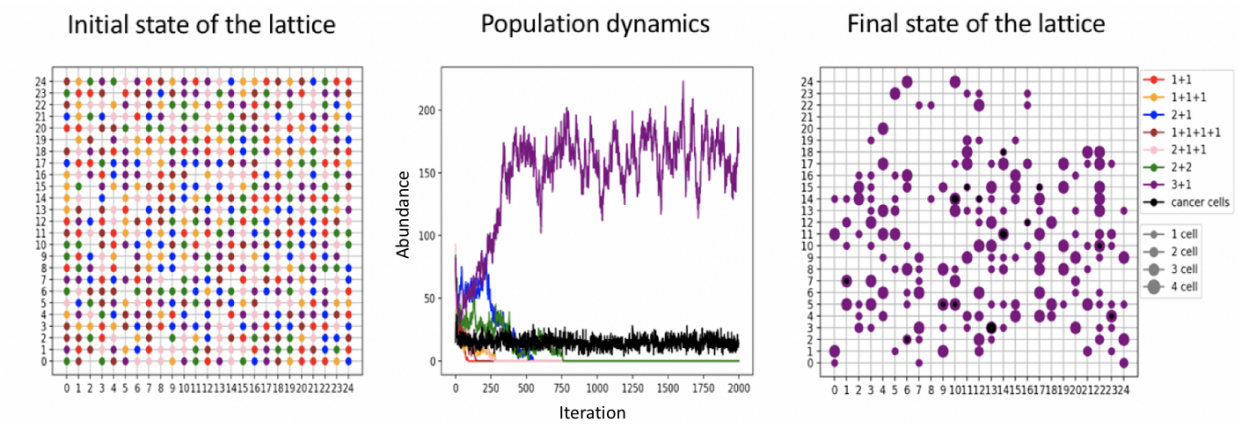

Figure 2. The initial state of the lattice, the abundances of each fragmentation mode (and the number of cancerous cells) throughout the simulation, and the lattice after 2000 timesteps. Parameters: T=2000, M=25, N_0=600, n=4, b=0.3, d=0.03, r=2, m=0.2, s=1, a=0.

Parameter	Description
M	The dimensions of the lattice
N_0	The initial population size
L	The size at fragmentation, i.e., adult size
n	The maximum adult size in a given simulation
b	The baseline rate of cell division
b_i	The division rate of cooperator cells in a group of i cooperators
B_i	The probability a group of size i with j cooperator cells grows by one cooperator cell
r	The replicative advantage of cancer cells

c_i	The division rate of cancer cells in a group of *i* cooperators
C_{ij}	The probability a group of *i* cooperators and *j* cancer cells grows by one cancer cell
d	The group death rate due to background mortality
D	The probability of group death due to background mortality
P	The proportion of cancer cells and the probability of group death due to cancer
s	Determines the degree of size dependent versus random competition
T	The number of iterations in the simulation
m	The probability a daughter cooperator cell mutates to a cancer cell

Table 2. A description of the parameters used in the simulation.

Results

Dominant modes in the absence of cancer

Our model explores a combination of features that has not been considered previously. To isolate the effect of each feature we first ignore cancer (*m*=0) and focus on the effect of competition under different modes of dispersal. To do this we run simulations with either random competition, where a fragment has a 50% chance of winning an occupied spot regardless of size (*s*=0), or size dependent competition, where the larger individual always wins the spot (*s*=1), and either global or local dispersal. For each combination of competition and dispersal we run simulations across a range of birth rates with max adult size *n*=4 and record the modes with the highest mean abundance (Figure 3; compare with figure 3A in Pichugin et al. 2017, where there is no competition).

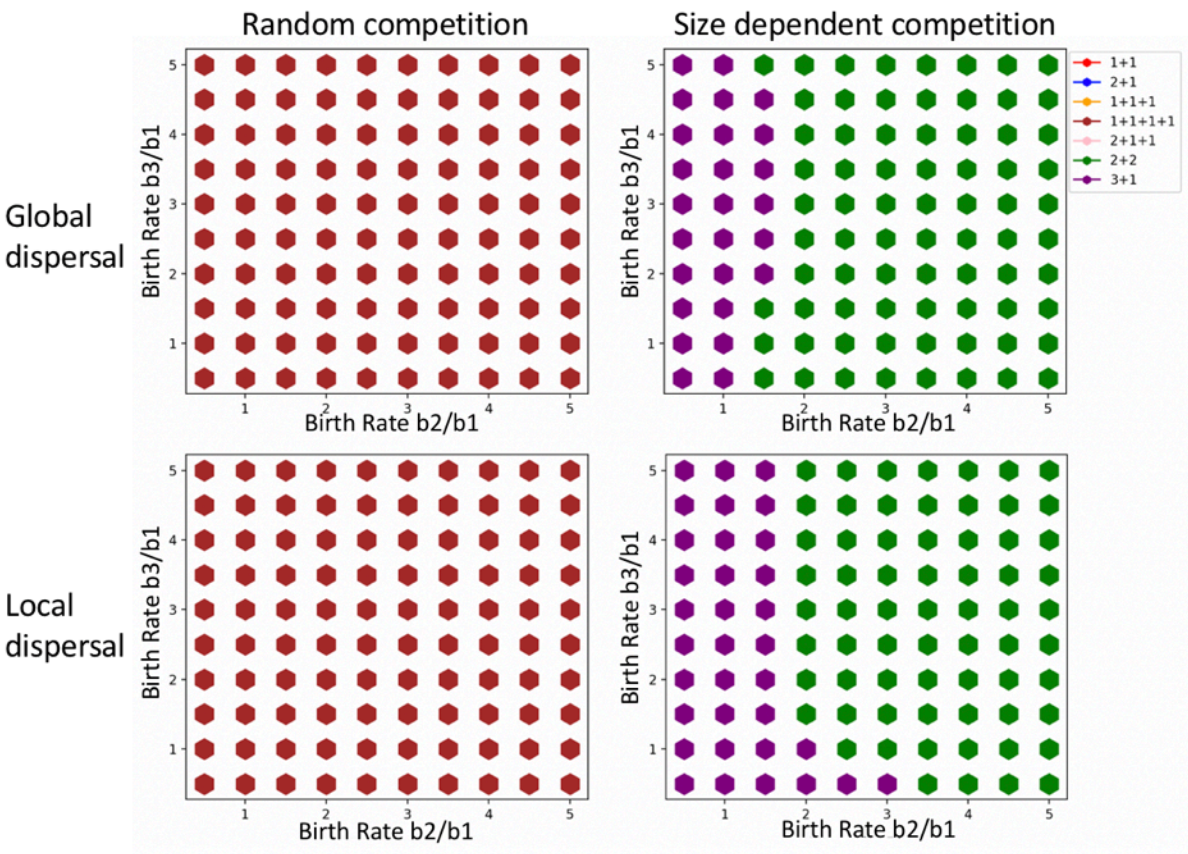

Figure 3. Dominant fragmentation modes in the absence of cancer (m=0). The dominant mode was the mode with the highest average abundance over 100 replicate simulations. Parameters: T=1000, M=50, N_0=2000, n=4, b_1=1, d=0, s=0 (left) and s=1 (right).

The left column of Figure 3 shows that random competition always favours multiple fission (1+1+1+1), regardless of birth rates or the mode of dispersal. What matters most in this case is the number of offspring per fragmentation event. It is perhaps surprising that the number of offspring per fragmentation event matters more than the number of offspring per time step (growth rate) in the absence of competition (see figure 3A in Pichugin et al. 2017). Our explanation is that multiple fission is effectively a bet-hedging

strategy. It has a lower expected growth rate than some other modes in the absence of competition but also has a lower variance in growth rate under random competition. In other words, having more slower growing offspring appears to be better, in this case, than having fewer faster growing offspring because having more offspring means that more offspring avoid being displaced by competition. This leads to multicellularity dominating despite being disfavoured by birth rates, i.e, $b_2 < b_1$ and $b_3 < b_1$. At first glance this seems to contradict the finding that binary fragmentation always wins under size-independent competition (Ress et al. 2022). However, a key difference is that in our simulation competition only happens during fragmentation, rather than continuously.

On the other hand, the right column of Figure 3 shows that size-dependent competition favours two binary fragmentation strategies: the unicellular propagule mode (3+1) and binary fission (2+2). When cells in groups of size 2 divide slowly (small b_2 values), 3+1 wins because offspring of size 3 do not have to go through this slow stage. For larger b_2 values 2+2 wins because groups of size 2 grow faster than groups of size 1. These results, especially under global dispersal, closely match those of Pichugin et al. (2017) (compare the top right panel of Figure 3 with their figure 3A), despite the fact they do not model competition. It is difficult to compare our results to theirs more quantitatively due to differences in the models (e.g., discrete vs. continuous time), but it appears that size-dependent competition may restrict the parameter space over which modes with smaller adult size win (e.g., Pichugin et al. (2017) see 1+1 or 2+1 win when $b_3 < b_1$).

It is interesting that the dominant modes under size-dependent competition (right panel of Figure 3) are more similar to the dominant modes in the absence of competition (Pichugin et al. 2017 figure 3A) than the dominant modes under random competition (left panel of Figure 3). This may be explained by the fact that the two strategies with the highest growth rate in the absence of competition over most of the parameter space (2+2 and 3+1) also have the largest possible adult (4) and mean offspring (2) sizes and therefore do well under size-dependent competition.

Under size-dependent competition, local dispersal allows the 3+1 mode to dominate in more of the parameter space (compare right panels in Figure 3). This might result from the fact that the 3+1 mode has two very different offspring sizes. Under local dispersal and size-dependent competition, the increased competition within each mode (intraspecific competition) will favour the larger offspring, which will displace the smaller. This means that 3+1 loses fewer cells to intraspecific competition than 2+2 does, giving it a growth advantage. It also means that the average size of surviving 3+1 offspring will be larger than 2, giving it an advantage in interspecific competition as well.

Dominant modes in the presence of cancer

After isolating the effect of size dependent competition and its interaction with dispersal, we now combine this with mutations to cancerous cells. Instead of varying birth rates continuously we choose two different scenarios (“size independent birth”, $a=0$, and “size dependent birth”, $a=1$) and vary the rate at which cells become cancerous (m). For each

parameter set we record the abundance of all modes at the end of the simulation and average over replicates (Figure 4).

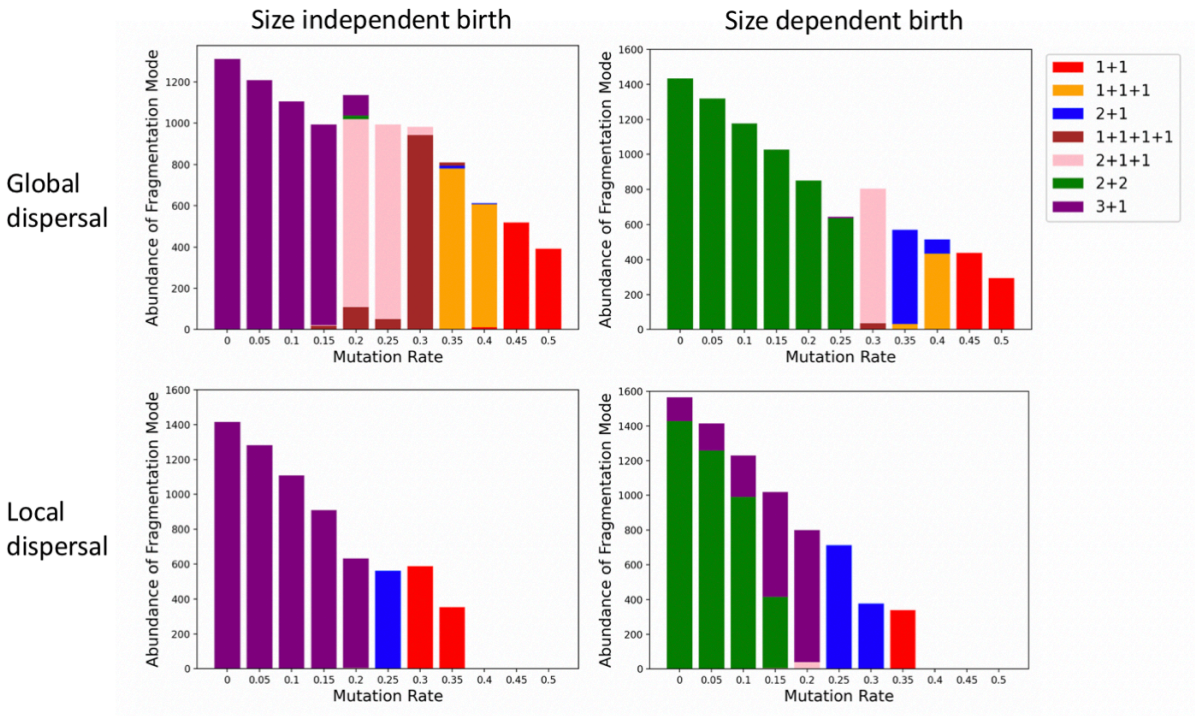

Figure 4. The abundance of each fragmentation mode as a function of the mutation rate to cancer. Abundances are averaged over 100 replicates. Parameters: T=5000, M=50, N_0=2000, n=4, b=0.3, d= 0.03, r=2, s=1.

Under size-independent birth and global dispersal (top left panel of Figure 4) we see the 3+1 mode winning in the cases of little to no cancer, which matches our earlier result ($b_2/b_1=b_3/b_1=1$ in the top right panel of Figure 3). As we increase the mutation rate we see a progression of the most abundant modes from 3+1, 2+1+1, 1+1+1+1, 1+1+1, to 1+1. This progression shows that modes with more single celled offspring are selected for by cancer. In particular, multiple fission is the best strategy for high mutation rates.

This is in line with the literature: unicellular bottlenecks purge lineages of cancer cells by aligning the fitness interests of the cell and the group. Only once the mutation rates are very high (which may not be biologically reasonable even during early multicellularity) do we see a decline in the optimal adult size. This decline in adult size may be because individuals are too likely to die of cancer before fragmenting.

Next we look at size-dependent birth rates and global dispersal (top right panel of Figure 4). Now cells in larger groups have a higher probability of dividing, e.g., because cells in a group can absorb and store resources more efficiently (Kaiser 2001). Binary fission (2+2) is the winning strategy with little or no cancer, consistent with previous results ($b_2/b_1 \cong 2$ and $b_3/b_1 \cong 3$ in the top right panel of Figure 3). As we increase the mutation rate we see a progression in the most abundant mode from 2+2, 2+1+1, 2+1, 1+1+1, to 1+1. As under size-independent birth, this is an increase in the number of unicellular offspring and eventually a decline in adult size. However, because solitary cells divide slowest under our size-dependent birth scenario, we do not see some multiple fission modes (1+1+1+1) and instead the initial large binary fragmentation mode (2+2) dominates at relatively high mutation rates.

Local dispersal, and the resulting increase in intraspecific competition, increases the advantage of the unicellular propagule mode over multiple fission under both birth rate

326 scenarios (bottom panels of Figure 4). In fact, only the unicellular propagule mode is
327 observed under size-independent birth (bottom left panel of Figure 4). The increased
328 intraspecific competition also causes a more precipitous drop in total population size
329 and the extinction of all modes at lower mutation rates.

330

331 *Mutation load*

332 To understand why particular modes dominate in the presence of cancer we run
333 simulations with one mode on the lattice at a time and calculate the proportion of cells in
334 the final population that are cancerous (Figure 5).

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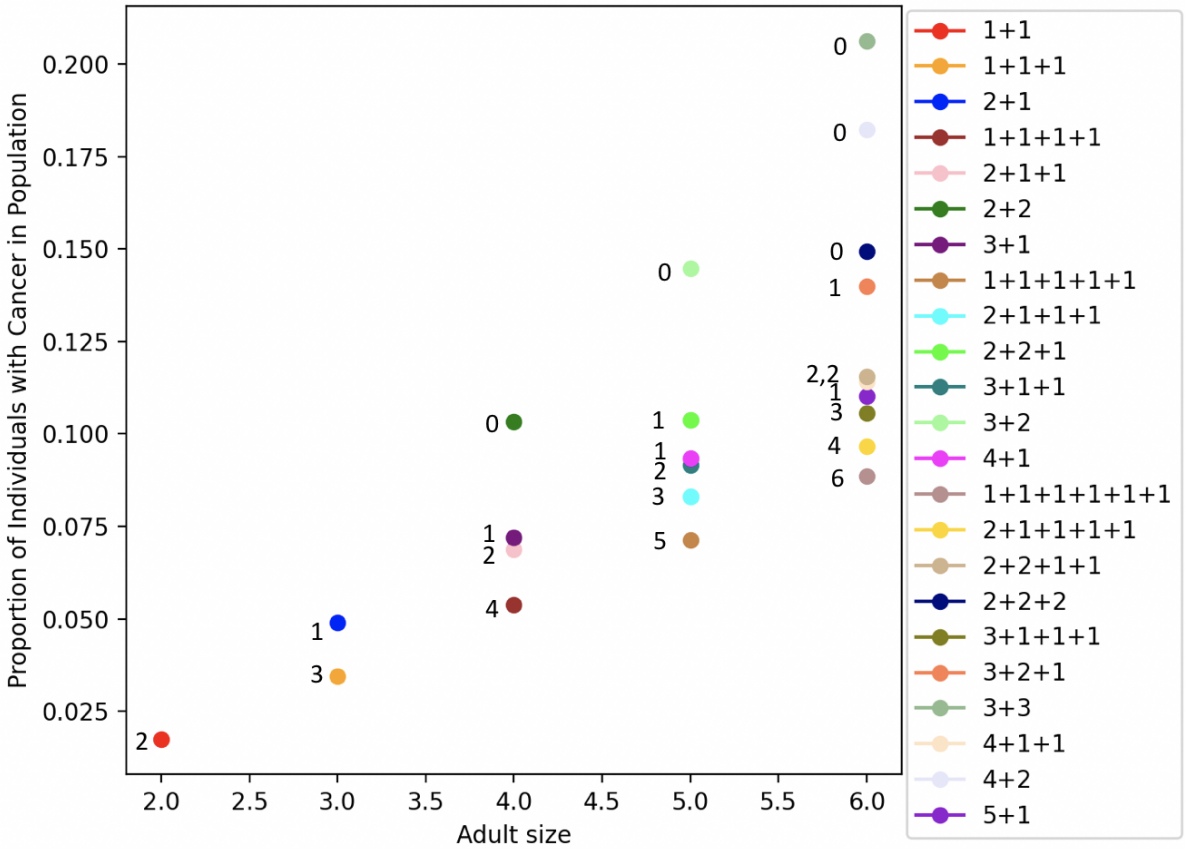

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Figure 5. Mutation load as a function of adult size, with numbers indicating the number of unicellular offspring in each mode. Parameters: T=1000, M=50, N_0=2000, n=6, b=0.3, d=0.03, r=2, m=0.2, s=1, a=0, global dispersal.

In Figure 5, for each adult size we see that multiple fission (1+1+…+1) has the lowest amount of cancer and binary fission has the highest. Moreover, the number of unicellular offspring does a very good job at ranking the mutation load of each mode. This sheds light on the patterns in Figure 4 – modes with more unicellular offspring have less cancer in their population because solitary cancer cells die with certainty.

An interesting outlier in Figure 5 is the largest unicellular propagule mode, 5+1. This mode is more effective at purging cancer than we would expect from the number of unicellular offspring. What may be going on here is that, since the cost of cancer is modelled as a death rate that is equal to the proportion of cancer in the group, lineages of large offspring are unlikely to survive for long since they are likely to have accumulated many cancerous cells. Therefore, populations of the 5+1 mode may propagate primarily through lineages of unicellular offspring. This is one hypothesis for why having all unicellular offspring or an extreme differential between offspring sizes keeps the cancer burden lower than having offspring of intermediate sizes.

Discussion

By simulating competition between both cells (cancer) and organisms in a spatial context we have found a number of interesting results that shed light on the evolution of early multicellular life cycles. First, we found that in the absence of cancer size-

independent (“random”) competition favours multiple fission, the strategies with the most offspring, while size-dependent competition favours binary fragmentation, the strategies with the largest average offspring (Figure 3). Second, the effect of cancer depends on the dispersal mode (Figure 4). Under global dispersal, increasing the rate of cancerous mutation first favours modes with more unicellular offspring (such as multiple fission), then modes with smaller adult sizes. Under local dispersal, the “unicellular propagule strategy” (Pichugin et al. 2017) is favoured at all rates of cancer, and increasing the rate only decreases adult size. Therefore, whether cancer can be said to explain the preponderance of species with the unicellular propagule strategy will depend on the nature of dispersal. Third, we explored the mechanism behind these trends and found that the number of unicellular offspring is negatively correlated with a population’s cancer burden under global dispersal (Figure 5), explaining the dominant modes.

One of our key new results is the dominance of life cycles other than binary fragmentation. Previous models have found binary fragmentation to dominate whether considering discrete or continuous time (Pichugin and Trauslen, 2022), exponential or frequency-dependent growth (Pichugin et al. 2017; Ress et al. 2022), or even when considering bidirectional switching between two cell types (Gao et al. 2017). Here, however, we find modes that produce multiple offspring to dominate under a wide range of conditions. For one, simply adding random competition between individuals causes multiple fission to dominate (Figure 3). We hypothesize this to be a bet-hedging strategy with a lower mean and variance in population growth rate, as compared to binary fragmentation, because multiple fission produces more but slower growing offspring. Adding cancer can also cause multiple fission to dominate (Figure 4). In this case the

reason appears to be because unicellular offspring are an effective way for an individual to purge cancer from its descendants (Figure 5). This is again a bet-hedging strategy – effectively culling some offspring so that others are cancer-free. One of the few factors previously found to promote multiple fission was costly fragmentation, where one cell is chosen to die at fragmentation (Pichugin et al. 2017). Curiously, this, like random competition and cancer, involves cell death. The other factor previously shown to favour multiple fission is a cost to groups of heterogeneous cells (Gao et al. 2019), which is very similar to cancer in our model. In nature, binary fragmentation is common. For example, *Trichoplax adhaerens* reproduces via binary fission and bacterial biofilms reproduce via the unicellular propagule strategy (Pichugin and Trauslen 2022; Ratcliff et al. 2017). But other fragmentation modes are also found. For example, species in the genera *Chlamydomonas, Volvox*, and *Gonium* reproduce via multiple fission into many single cells (Hanschen et al. 2016; Pichugin and Trauslen 2022). It would be interesting to know if the factors promoting non-binary strategies in our model (the nature of competition and/or the prevalence of cancer) can help explain their existence in nature.

By simulating both global and local dispersal we identified the effect of spatial structure and its interaction with cancer. In the absence of cancer, local dispersal increased the parameter space over which the unicellular propagule mode dominated binary fission (Figure 3). We reason this to be due to increasing the relative strength of intraspecific (vs. interspecific) competition. Given competition is size dependent, intraspecific competition will disfavour smaller offspring. This means that the unicellular propagule mode will mostly lose unicellular offspring, which have a lower reproductive value than the larger offspring. Since the two offspring under binary fission are equivalent,

intraspecific competition causes a larger loss of reproductive value in this mode. With cancer, local dispersal prevents modes with more than two offspring from dominating (Figure 4). Our reasoning for this is similar: having more offspring means dividing reproductive value into more, and hence more equal, pieces. Given intraspecific competition for space, more offspring means more death, and therefore a larger loss of reproductive value. By preventing multiple fission from dominating and, more generally, by decreasing the relative fitness of unicellular offspring, local dispersal hinders the purging of cancer.

Throughout much of parameter space, increasing rates of cancer favours multiple fission (Figure 4) as it is the most efficient mode at purging cancer (Figure 5). This supports our hypothesis that cancer will select for life cycles with unicellular offspring. On the other hand, it also forces us to clarify what we mean when we say that cancer selects for ‘the unicellular strategy’. It is often implied that humans, like many metazoans, partake in the unicellular strategy, since all offspring start from a unicellular zygote. However, in a model of overlapping generations, like ours, (ignoring the complications of sex) humans would use something like the unicellular propagule mode with a very large adult size: reproduction creates one unicellular offspring and one surviving adult. More generally, the unicellular bottleneck is often synonymous with the unicellular propagule strategy (Grosberg and Strathmann 2007; Grosberg and Strathmann 1998; Marquez-Zacarias et al. 2021; Avise 1993). Here we have shown that, while the unicellular propagule strategy is more effective at minimizing cancer burden than some other modes, such as binary fission, multiple fission is the most effective. Of course, the costs and benefits of the unicellular bottleneck are experienced

427 by any life cycle that has at least one unicellular offspring. We therefore suggest the
428 unicellular strategy be thought of as a continuum, with some modes using more of this
429 strategy than others, and with multiple fission being one extreme. As we have shown,
430 the optimal amount of the unicellular strategy to use will depend on a variety of factors,
431 such as the nature of competition and the rate of mutation to cancerous cells. Finally,
432 we note that many previous models have assumed non-overlapping generations (e.g.,
433 Roze and Michod 2001), in which case cancer selects for a large adult creating many
434 unicellular offspring, reminiscent of multiple fission.

435 One limitation of our model is that the number of fragmentation modes grows rapidly
436 with adult size, making it computationally tedious to explore. Intuitively, increasing adult
437 size increases the number of cellular generations per organismal generation. This
438 increases the strength of selection among cells relative to selection among organisms –
439 resulting in larger cancer loads for a given number of unicellular offspring (Figure 5).
440 When scaling up our simulation to larger adult sizes there are two limitations to keep in
441 mind: 1) we assume each group can grow by at most one cell of each type per timestep
442 and 2) we assume there are only 5 spots available for offspring (above, below, right, left
443 or on the parent's spot) under local dispersal. Both limitations will disadvantage larger
444 adult sizes and should therefore be relaxed for a fair analysis.

445 Returning to the bigger picture, concerning evolutionary transitions in individuality and
446 multilevel selection - cancer is a problem resulting from discordant selection. Units of
447 selection are built on top of existing units and when these units have different time
448 scales (e.g., organismal generation >> cellular generation) there is the opportunity for
449 short-sighted selection to disrupt cooperation within the larger unit. Despite this issue,

multicellularity has been an incredibly successful and widespread strategy that has persisted for millions of years. So how has this problem of discordant selection been dealt with? Here we explored one such hypothesized adaptation – the unicellular propagule. Our model tells us that unicellular propagules are an efficient way to purge cancer and are selected for even in the presence of competition that favours larger organisms. In this way, our model adds weight to the argument that the unicellular bottleneck may have helped facilitate multicellularity at its origins.

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