Dynamic cell differentiation in multicellularity with specialized cell types

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Abstract

The specialization of cells is a hallmark of complex multicellularity. Cell differentiation enables the emergence of specialized cell types that carry out segregated functions which are previously executed by a multifunctional ancestor cell. Yet, it is still unclear why cell differentiation evolved in the first place, especially for genetically identical cells, exposed to the same life history environment. Stochasticity in gene expression has been proposed to account for cell differentiation. We develop a theoretical model to investigate the effect of dynamic cell differentiation — cells can change their developmental trajectories during a single round of development — on the evolution of their cell differentiation strategy. We found that in small organisms, irreversible differentiation – a cell type gradually losing its cell differentiation capability to produce other cell types – classified based on the differentiation capability at the last cell division, is favoured over other differentiation strategies under dynamic cell differentiation. Dynamic cell differentiation allows a wide range of differentiation strategies, allowing more evolutionary possibilities than static cell differentiation.

Author summary

The differentiation of cells into different branches is a characteristic feature of multicellular organisms. To understand its origin, the mechanism of division of labour was introduced, where cells are specialized at distinct tasks. During the differentiation process, a cell type is usually assumed to produce another cell type with a fixed probability. However, it has been argued that cell differentiation is a dynamic process, where cells possess changing differentiation capabilities during an organism's development. How does dynamic differentiation impact the evolution of differentiation patterns? Here, we classified the patterns based on cells' differentiation capability in the last cell division and then investigated their effects on an organism's growth. We seek the "optimal strategy" which leads to the fastest growth analytically and numerically. Our results show that irreversible differentiation which gradually loses its differentiation capability, is favoured over static differentiation in small organisms. Dynamic differentiation exhibits a more diverse pattern of development than static differentiation, which can lead to novel targets of selection.

Introduction

The transition to multicellularity is one of the major evolutionary transitions for the evolution of life on earth and it is associated with the differentiation of cells into different cell types [1–5]. Especially large multicellular organisms possess diverse cell types for diverse functions [6–8]. It is widely accepted that multicellular life has evolved from simple unicellular ancestors [9, 10]. Cell differentiation enables a diversity of cell types, leading unicellular organisms to form increasingly larger and more complex organizations. Differentiated cells perform distinct functions in varying conditions and therefore can increase an organism's fitness. For example, cell differentiation occurs under adverse environmental conditions to increase an organism's survival chance, such as cyanobacteria differentiating nitrogen-fixing heterocysts under nitrogen-free conditions [11], or *Myxococcus xanthus* producing a new cell type under starvation [10]. The underlying mechanisms leading to the origin of cell differentiation are still unclear [12].

Several mechanisms have been proposed to understand cell differentiation and phenotypic variation, from the perspective of gene expression, mutations, epigenetics, and the environment [8, 9, 13–17]. These mechanisms are complementary, and thus, more than one mechanism could act during the evolution of cell differentiation of a species [14, 16]. These mechanisms usually assume that multifunctional and unicellular ancestors differentiate specialized cells to carry out segregated functions, even when cells are genetically identical and have been exposed to an identical environment. Stochasticity in gene expression, leading cells to have different extents or even loss of cell functions, is one of the mechanisms to understand why genetically identical cells show significant phenotypic variation [8, 16, 18, 19]. Stochasticity in gene expression may be caused by the inherently random biochemical reactions at single-cell level [18–21]. This randomness makes cell types dynamically change during an organism's growth, leading to dynamic developmental trajectories. So far, there is insufficient theoretical understanding of the effects of dynamic developmental trajectories on the evolution of cell differentiation strategies.

Studies of cell differentiation have mainly focused on the optimal condition, where mature cells of an organism allocate their ressources to different tasks [22–33]. Essentially, they are focused on the proportion of each cell type in an organism, instead of the stochastic developmental process of each cell type during an organism's growth. Cells capable of switching to another type have not been in the focus yet. Some authors considered cell differentiation abilities, but only in one cell type while other cell types were terminally differentiated types (without division ability) [23, 25, 28]. Rodrigues et al. considered cell differentiation ability as an evolving trait, but the trait was coupled with varying cell division rates and organisms were constrained in filament form [34]. More recently, Cooper et al. introduced a random specialization model, but the random process only impacts the final fractions of different cell types rather than the internal organization of task allocation of cells during an organism's growth [33]. Gavrilets and Gao et al. considered cell differentiation between cell types, but differentiation probabilities are assumed to be static rather than dynamic [24, 35]. So far, little is known about the effects of dynamic strategies on the evolution of cell differentiation.

In this study, we develop a theoretical model to investigate the effect of dynamic cell differentiation on the population growth rate. Here, dynamic cell differentiation refers to the capability of having different cell differentiation probabilities for a type of cell between any two successive cell divisions. Comparatively, static differentiation only allows a fixed cell differentiation probability for a type of cell across cell divisions [35]. We consider two cell types: cells capable of producing a new organism (germ-like) and cells that speed up growth (soma-like). For convenience, we classify cell differentiation strategies only by the differentiation probabilities in the last cell division, where we

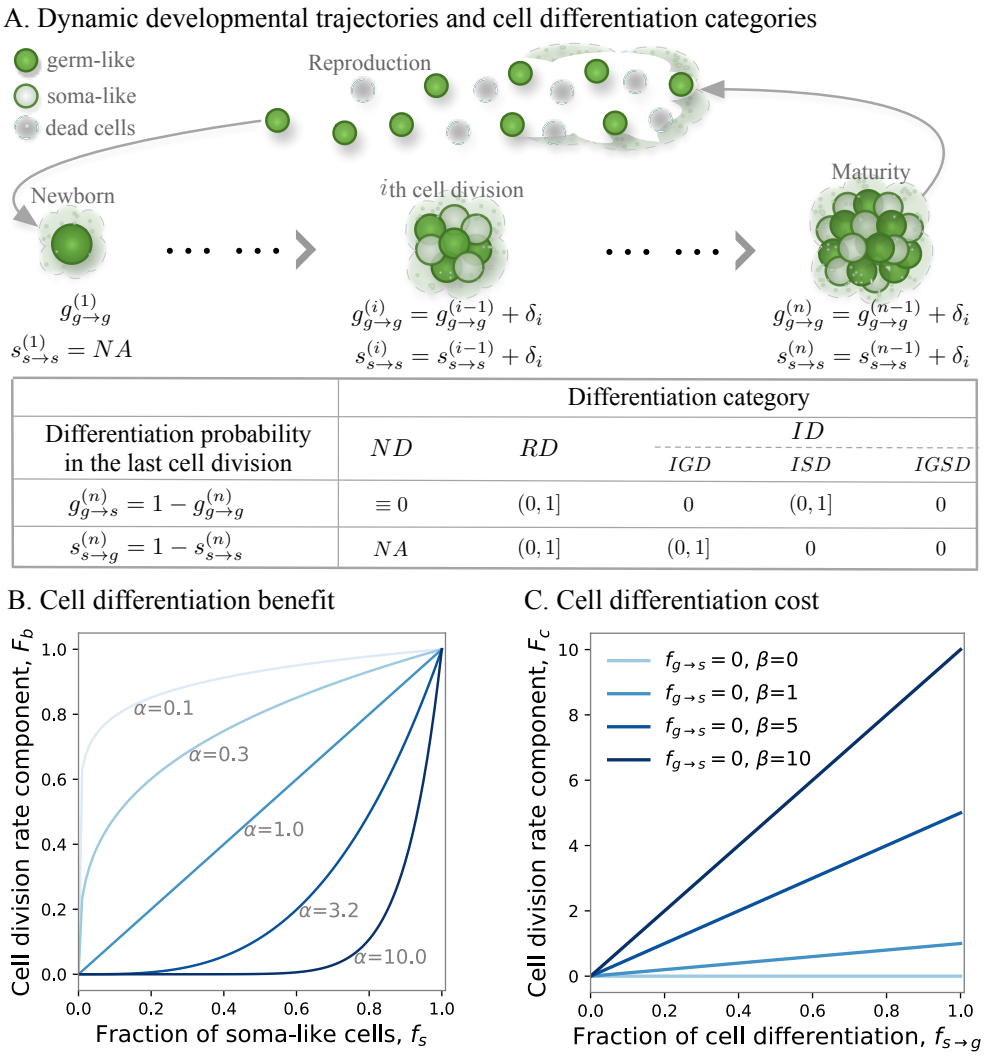
work with three categories: non-differentiation ND, reversible differentiation RD, and irreversible differentiation ID. Irreversible differentiation (ID) includes three subcategories: irreversible germ differentiation IGD, irreversible soma differentiation ISD, and irreversible germ and soma differentiation $IGSD$. We should note that each of our categories includes many cell differentiation strategies, except the category ND. Populations have different cell differentiation strategies and compete with each other via the resulting population growth rate. We assume that an organism's growth (in terms of the number of cells) depends on the fraction of soma-like cells and transition probabilities between cell types. The optimal strategy is the one leading to the fastest population growth. We compute the optimal strategy in different parameter spaces and compare the evolutionary differences of optimal strategies under dynamic and static cell differentiation.

Model and methods

We use population growth rate as a proxy to investigate the evolution of different cell differentiation strategies. We assume that populations differ in their cell differentiation strategies and compete for growth. Each population starts with an organism and its offspring adopt the same dynamic cell differentiation strategy. Each organism starts with a single germ-like cell, see Fig 1A. Cells divide synchronously, each cell producing two daughter cells at a time. After the ith cell division, organisms have 2^i cells in total. Organisms grow and mature until they reach a maturity size 2^n, where n is the maximal cell division of organisms. Mature organisms release germ-like cells as offspring and soma-like cells die. For each division, cells have a set of probabilities to produce daughter cells of a certain type. $g_{gg}^{(i)}$ is the probability of a germ-like cell producing two germ-like cells at the ith cell division. $g_{gs}^{(i)}$, $g_{ss}^{(i)}$, $s_{gg}^{(i)}$, $s_{gs}^{(i)}$ and $s_{ss}^{(i)}$ are defined in a similar manner, where we have $g_{gg}^{(i)} + g_{gs}^{(i)} + g_{ss}^{(i)} = 1$ and $s_{gg}^{(i)} + s_{gs}^{(i)} + s_{ss}^{(i)} = 1$ for each growth stage i, $i = 1, 2, \ldots, n$. We denote $d_i = [g_{gg}^{(i)}, g_{gs}^{(i)}, g_{ss}^{(i)}, s_{gg}^{(i)}, s_{gs}^{(i)}, s_{ss}^{(i)}]$ as the cell differentiation probabilities in the ith cell division. In addition, $g_{g\to s}^{(i)} = \left(g_{ss}^{(i)} + \frac{g_{gs}^{(i)}}{2}\right)$ and $s_{s\to g}^{(i)} = \left(s_{gg}^{(i)} + \frac{s_{gs}^{(i)}}{2}\right)$ are referred to as transition probabilities, $i = 1, 2, \ldots, n$. The cell differentiation probabilities across the successive n times of cell divisions of an organism can be expressed in matrix form as

$$\mathbf{D} = \begin{pmatrix} d_1 \\ \vdots \\ d_i \\ \vdots \\ d_n \end{pmatrix} = \begin{pmatrix} g_{gg}^{(1)} & g_{gs}^{(1)} & g_{ss}^{(1)} & s_{gg}^{(1)} & s_{gs}^{(1)} & s_{ss}^{(1)} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ g_{gg}^{(i)} & g_{gs}^{(i)} & g_{ss}^{(i)} & s_{gg}^{(i)} & s_{gs}^{(i)} & s_{ss}^{(i)} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ g_{gg}^{(n)} & g_{gs}^{(n)} & g_{ss}^{(n)} & s_{gg}^{(n)} & s_{gs}^{(n)} & s_{ss}^{(n)} \end{pmatrix}, \tag{1}$$

where the ith row of the matrix contains the cell differentiation probabilities in the ith cell division. We call $\mathbf{D}$ dynamic cell differentiation, as cell differentiation probabilities can change between differentiation steps. We assume that this change is not larger than δ, e.g. $g_{g\to s}^{(i)} = g_{ss}^{(i)} + \frac{g_{gs}^{(i)}}{2} = g_{g\to s}^{(i-1)} \pm \delta_i$ with δ sufficiently small such that all probabilities remain well defined. If $\delta_i = 0$, $i = 1, 2, \ldots, n$, then $\mathbf{D}$ is a static cell differentiation strategy, where the same type of cells follow a fixed set of probabilities to produce daughter cells at each division. We should note that static cell differentiation is defined by the values of $g_{g\to s}^{(i)}$ and $s_{s\to g}^{(i)}$, thus the same static cell differentiation could have different values of $g_{ss}^{(i)}$ and $g_{gs}^{(i)}$ ($s_{gg}^{(i)}$ and $s_{gs}^{(i)}$). In this work, the acronyms of



Differentiation probability in the last cell division	Differentiation category				
	ND	RD	ID		
			IGD	ISD	$IGSD$
$g^{(n)}_{g\to s} = 1 - g^{(n)}_{g\to g}$	$\equiv 0$	$(0, 1]$	0	$(0, 1]$	0
$s^{(n)}_{s\to g} = 1 - s^{(n)}_{s\to s}$	NA	$(0, 1]$	$(0, 1]$	0	0

Fig 1. Illustration of dynamic cell differentiation, differentiation strategies, and cell division rate components. A. Schematic of an organism's life cycles and dynamic cell differentiation strategies. Organisms start from single germ-like cells and undergo n synchronous cell divisions before reproduction. For newborn organisms, as there are no soma-like cells, the cell differentiation probability for soma-like cells is irrelevant. Cell differentiation probabilities can change from the $(i-1)$th cell division to the ith cell division by a small quantity δ_i ($0 \le \delta_i \le 1$ and $i = 1, 2, \ldots, n$). Based on the cell differentiation probabilities, we classify cell differentiation into three categories: non-differentiation ND, reversible differentiation RD, and irreversible differentiation ID. For ND, we have $g^{(i)}_{g\to g} = 1$, $i = 1, \ldots, n$. **B.** The benefits of cell differentiation. We assume that the cell division rate increases with the fraction of soma-like cells f_s. For the associated benefit, we assume $F_b = b(f_s)^\alpha$, where the shape of the function is controlled by α. **C.** The costs of cell differentiation. We assume that the cell division rate decreases with the fraction of cell divisions that turn a soma-like cell into a germ-like cell and viceversa. For the associated cost, we assume $F_c = c(f_{g\to s} + \beta f_{s\to g})$. Here, we show the values of F_c with varying $f_{s\to g}$ and β by setting $f_{g\to s} = 0$ (Parameters: $b = 1$ in panel **B** and $b = 1$ in **C**).

differentiation strategies are dynamic unless otherwise stated. Different cell differentiation strategies lead to different differentiation degrees. For example, if a

strategy has $g^{(i)}_{g\to s} = 0$ for $i = 1, 2, \ldots, n$, then organisms have no cell differentiation. If a strategy has $g^{(i)}_{g\to s} = 1$ and $s^{(i)}_{s\to g} = 1$, then organisms have maximal cell differentiation.

We assume that cell differentiation impacts an organism's growth [35]. The effects of cell differentiation are further decomposed into cell differentiation benefits and costs on growth. We assume differentiated soma-like cells are beneficial and increase an organism's growth. Cell differentiation between germ-like cells and soma-like cells is costly and decreases growth. Organisms grow faster with higher cell division rates and vice versa. Specifically, $r^{(i)}$ represents the growth rate in the ith cell division and is determined by two components

$$r^{(i)} = \frac{1 + F_b^{(i)}}{1 + F_c^{(i)}}, \tag{2}$$

where $F_b^{(i)}$ and $F_c^{(i)}$ are the effects of cell differentiation benefit and cell differentiation cost in the ith cell division, $i = 1, 2, \ldots, n$. F_b is a function of the fraction of soma-like cells f_s,

$$F_b = b(f_s)^\alpha, \tag{3}$$

where the b is the benefit scale, $b \geq 0$. α controls the shape of the function, see Fig 1B. F_c is a function of the fraction of cell differentiation between germ-like cell and soma-like cell $f_{g\to s}$ and $f_{s\to g}$,

$$F_c = c(f_{g\to s} + \beta f_{s\to g}), \tag{4}$$

where c is the cost scale, $c \geq 0$. β measures the relative cost of differentiation from soma-like cell to germ-like cell, see Fig 1C. The fractions of cell differentiation in the ith cell division are

$$\begin{aligned} f^{(i)}_{g\to s} &= f^{(i-1)}_g g^{(i)}_{g\to s} \\ f^{(i)}_{s\to g} &= f^{(i-1)}_s s^{(i)}_{s\to g}, \end{aligned} \tag{5}$$

where $f^{(i-1)}_g$ and $f^{(i-1)}_s$ are the fraction of germ-like cell and soma-like cell after the $(i-1)$th cell division, respectively. Note that $f^{(i-1)}_g + f^{(i-1)}_s = 1$, $g^{(i)}_{g\to g} + g^{(i)}_{g\to s} = 1$, and $s^{(i)}_{s\to s} + s^{(i)}_{s\to g} = 1$, $i = 1, 2, \ldots, n$. Specifically, $f^{(i)}_g$ and $f^{(i)}_s$ are calculated by using transition probabilities, see Eq (11) in S1 Appendix. Taking Eq (2), Eq (3), Eq (4) and Eq (5) together, we have

$$r^{(i)} = \frac{1 + b(f^{(i-1)}_s)^\alpha}{1 + c(f^{(i)}_{g\to s} + \beta f^{(i)}_{s\to g})}. \tag{6}$$

After the $(i-1)$th cell division, the waiting time before the ith cell division occurring $t^{(i)}$ follows the exponential distribution $f(t^{(i)}) = r^{(i)} e^{-r^{(i)} t^{(i)}}$, $i = 1, 2, \ldots, n$. Thus the expected waiting time from the $(i-1)$th to the ith cell division is $t^{(i)} = \frac{1}{r^{(i)}}$ [36]. The expected growth time of organisms with n cell divisions is

$$t = \sum_{i=1}^{n} t^{(i)} = \sum_{i=1}^{n} \frac{1}{r^{(i)}} = \sum_{i=1}^{n} \frac{1 + c\left(f^{(i-1)}_g g^{(i)}_{g\to s} + \beta f^{(i-1)}_s s^{(i)}_{s\to g}\right)}{1 + b\left(f^{(i-1)}_s\right)^\alpha}. \tag{7}$$

We consider a density-independent population, see [37] for a discussion when density dependence is relevant in a related model. The population growth rate of an organism only depends on the number of offspring and their growth time. As organisms divide

synchronously, the number of offspring that an organism produces during its life is $2^n f_g^{(n)}$. Therefore, organisms grow exponentially and the population growth rate is

$$\lambda = \frac{\ln N}{t} = \ln(2^n f_g^{(n)}) \sum_{i=1}^{n} \frac{1}{r^{(i)}} = \ln\left(2^n f_g^{(n)}\right) \sum_{i=1}^{n} \frac{1 + c\left(f_g^{(i-1)} g_{g\to s}^{(i)} + \beta f_s^{(i-1)} s_{s\to g}^{(i)}\right)}{1 + b\left(f_s^{(i-1)}\right)^{\alpha}}, \tag{8}$$

where n is the number of cell division an organism undergoes before maturity. $f_g^{(i)}$ and $f_s^{(i)}$ are fractions of germ-like cell and soma-like cell after the ith cell division. Here, $g_{g\to s}^{(i)}$ and $s_{s\to g}^{(i)}$ are the transition probabilities between germ-like cell and soma-like cell in the ith cell division ($1 \le i \le n$), see the S1 Appendix. As each organism starts with a single germ-like cell, $f_g^{(0)} = 1$ and $f_s^{(0)} = 0$. If a strategy has no cell differentiation i.e. $g_{gg}^{(i)} = 1$, we call it ND, $i = 1, 2, \ldots, n$. In ND, cells only produce germ-like cells, thus $g_{g\to g}^{(i)} = 1$ for all i, and all other probabilities are irrelevant. Therefore, $f_g^{(i)} \equiv 1$, $f_s^{(i)} \equiv 0$. From Eq (8), the population growth rate of ND is $\lambda_{ND} = \ln 2$. Biologically, it means that organisms double their size per unit of time when they take the ND strategy.

Results

Dynamic cell differentiation includes more types of cell differentiation strategies

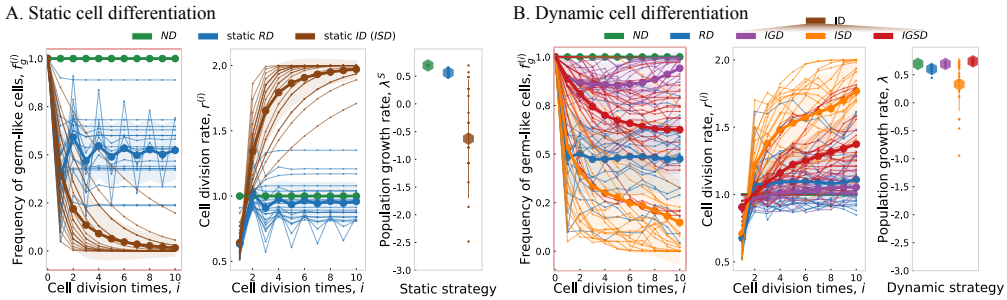


Fig 2. A. Population growth rate of static cell differentiation. Frequencies of the germ-like cells, cell division rates, and population growth rates of randomly chosen strategies in each category of static differentiation, ND, static RD, and static ID. **B. Population growth rate of dynamic cell differentiation.** Randomly chosen strategies in each category of dynamic differentiation: ND, RD, and ID (IGD, ISD, and $IGSD$). Static ISD is the only subcategory in static ID. We should note that only the fraction of germ-like cells after the last division $f_g^{(n)}$ and the growth time contribute to the population growth rate. Thick dots and shaded areas (error bars in the last panels) are the averaged values and the standard deviation of the strategies in each category, respectively. Parameters in both panels: $n = 10$, $b = c = 1$, $0 \le \delta \le 0.1$. We chose 20 random strategies in each category and then recorded the frequency of germ-like cells, the cell division rates after each cell division, together with their final population growth rates (thin lines). For the numerical calculation of the population growth rate, see S2 Appendix.

Compared with static differentiation, dynamic differentiation includes a broader spectrum of differentiation paths. In our model, we classify differentiation based on the probabilities in the last cell division for convenience. There are three categories: non-differentiation (ND), reversible differentiation (RD), and irreversible differentiation (ID), see Fig 1A. ND has the transition probabilities $g_{g\to s}^{(i)} = 0$, and thus there are no

transition probabilities $s^{(i)}_{s\to g}$, $i = 1, 2, \ldots, n$. By definition, ND is static. RD and ID both involve cell differentiation, but the difference is the transition probability value in the last cell division n. For RD, both cell types have non-zero transition probabilities in the nth cell division, i.e. $0 < g^{(n)}_{g\to s} < 1$ and $0 < s^{(n)}_{s\to g} < 1$. By contrast, at least one cell type in ID has 0 transition probability in the nth cell division, i.e. either $g^{(n)}_{g\to s} = 0$ or $s^{(n)}_{s\to g} = 0$. Static ISD is the only ID in static differentiation. Based on the two cell types in the model, ID can further be divided into three subcategories: irreversible germ differentiation (IGD, $g^{(n)}_{g\to s} = 0$, but existing at least one division makes $g^{(i)}_{g\to s} \neq 0$ for $i \neq n$), irreversible soma differentiation (ISD, $s^{(n)}_{s\to g} = 0$) and irreversible germ and soma cell differentiation ($IGSD$, $g^{(n)}_{g\to s} = s^{(n)}_{s\to g} = 0$), see Fig 1A. Static differentiation is a subset of dynamic differentiation [35].

Dynamic differentiation leads to higher standard deviations in the fraction of germ-like cells, cell division rate, and population growth rate than static differentiation, see Fig 2. Since cells with the same type divide with the same probabilities in static differentiation, the fractions of the germ-like cell of different categories approach stationary states over time, see Fig 2A. For example, the fractions of germ-like cells in a static RD approach a constant value, while the fractions in static ISD decrease monotonically to 0. However, the fractions of the germ-like cell of different categories under dynamic differentiation fluctuate irregularly and the patterns are more complex, see Fig 2B. For example, the fractions of germ-like cells have a minimum value in the middle of cell divisions in IGD on average. This pattern shows that IGD invests into soma-like cells first and later lets soma-like cells contribute to offspring i.e. produce germ-like cells. Compared with static ID, ID leads to three very different developmental trajectories, where IGD keeps higher fractions of germ-like cells, ISD has the lowest fractions and $IGSD$ has intermediate fractions. Moreover, we found that static ISD and ISD both lead to higher standard deviations in population growth rates. This is because static ISD and ISD contain many strategies without producing any offspring. Based on Eq (8), we know when $f^{(n)}_g < \frac{1}{2^n}$, then $\lambda < 0$.

Dynamic cell differentiation promotes irreversible cell differentiation in small organisms

Without constraints, the differences of cell differentiation probabilities in successive cell divisions can take any value, i.e. $0 \leq \delta_i \leq 1$. Then, an organism's growth and cell differentiation are completely arbitrary. An extreme example that optimally exploits the potential of somatic cells would be that both types of cells produce soma-like cells in the first $(n-1)$ divisions, and then all produce germ-like cells in the last division. We refer to this cell differentiation as "Extreme differentiation" (ED). ND is a strategy that does not receive cell differentiation benefit or cell differentiation cost. In contrast, ED is a strategy that fully uses cell differentiation benefit. Naturally, we expect that when $b \gg 1$ and $c \ll 1$, ED is optimal, and when $c \gg 1$ and $b \ll 1$, ND is optimal. Based on Eq (8), the population growth rate of ED is $\lambda_{ED} = \frac{\ln 2}{\frac{n+b+(1+b+\beta)c}{n(1+b)}}$ and the population growth rate of ND is $\lambda_{ND} = \ln 2$. Thus, when $c < \frac{(n-1)b}{1+b+\beta}$, we have $\lambda_{ED} > \lambda_{ND}$ and when $c > \frac{(n-1)b}{1+b+\beta}$, we have $\lambda_{ED} < \lambda_{ND}$, see Fig 3A. For $\lambda_{ED} = \lambda_{ND}$ and $0 \leq \delta_i \leq 0.1$, numerical results show that population growth rates dominance of differentiation strategies over ED and ND increase with cell differentiation benefits, especially for IGD (Fig 3B). Under the condition of $\lambda_{ND} \neq \lambda_{ED}$, we found that strategies lead to diverse population growth rates. This implies evolutionary diversity under dynamic differentiation with varying differentiation benefits and costs.

Next, we investigate the effects of dynamic differentiation on the population growth

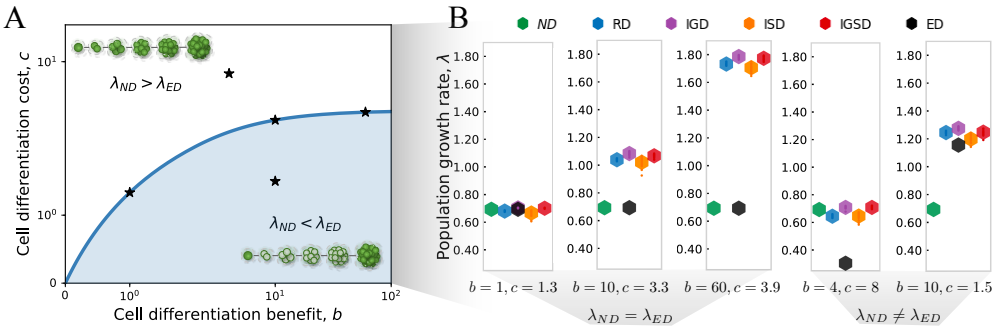


Fig 3. The evolutionary conditions for non-differentiation ND and extreme differentiation ED, and the population growth rates of dynamic differentiation strategies. A. Comparison of population growth rates between ND and ED. The blue line represents the condition for $\lambda_{ND} = \lambda_{ED}$. The shaded area represents where $\lambda_{ND} < \lambda_{ED}$. The black stars correspond to the parameter combinations investigated in panel **B**. **B.** Population growth rates of different strategies relative to $\lambda_{ND} = \lambda_{ED}$. The error bars indicate the standard deviation. Parameters: $n = 5$, $\alpha = \beta = 1$ and $0 \leq \delta_i \leq 0.1$. We generate 20 duplicates of optimal strategies in each category. For the numerical calculation of the population growth rate, see S2 Appendix.

rate under varying differentiation benefits and costs, comparing it with the results of static differentiation strategies. As it has been shown that cell differentiation is a complex process [38], it would be natural to assume that the maximum value of the change of two successive differentiation probabilities is small. Thus, we restrict attention to small range of δ_i and set $0 \leq \delta_i \leq 0.1$, $i = 1, \ldots, n$. We found that dynamic differentiation promotes the evolution of irreversible strategies ID compared with static differentiation in small organisms, see Fig 4. In the model, cell differentiation plays a dual role in population growth rate. It brings benefits, but also incurs costs on the population growth rate. The best strategy is the one that can maximally use cell differentiation benefits and at the same time reduce costs. Due to the randomness of cell differentiation probabilities, dynamic strategies can adjust the fraction of germ-like cells during growth. Thus, dynamic differentiation strategies (either RD or ID) can lead to higher population growth rates than static ones (either static RD or static ISD).

ND dominates under both static and dynamic differentiation at high differentiation costs as costs largely decrease the population growth rates of differentiation strategies. In the absence of differentiation benefits, i.e. $b = 0$ and $c > 0$, ND is optimal (S3 Appendix). Additionally, if there is only a single cell division ($n = 1$), ND is still optimal in the absence of differentiation costs, i.e. $c = 0$ and $b > 0$ (S3 Appendix) This is because differentiation benefits F_b are based on the fraction of soma-like cells after the previous division. However, we found that dynamic ID replaces static ND being the optimal strategy at small b and c, Fig 4A. Specifically, it is an IGD strategy replacing ND when b is slightly larger than c (Fig 4C and the first panel of Fig 4D). Small b and c have little but relatively the same effects on the population growth rate, see Eq (8). Thus, under this scenario, the best strategy would be the one producing a few soma-like cells at an early stage to obtain differentiation benefits, and then let the majority of cells produce their own cell types to reduce differentiation costs. Therefore, IGD with such characteristic becomes optimal, see the first panel in Fig 4D. Although $s^{(n)}_{s \to g}$ seems quite large in this optimal IGD strategy, the fraction of soma-like cells is very small $(1 - f_g)$. Thus, the IGD strategy bears lower differentiation costs. Furthermore, RD is optimal at relatively large b and small c. In this scenario, the effects of b on the population growth rate overweight the effects of c. Thus, even though RD has high cell transition probabilities, its high cost can be overcome by its differentiation benefits.

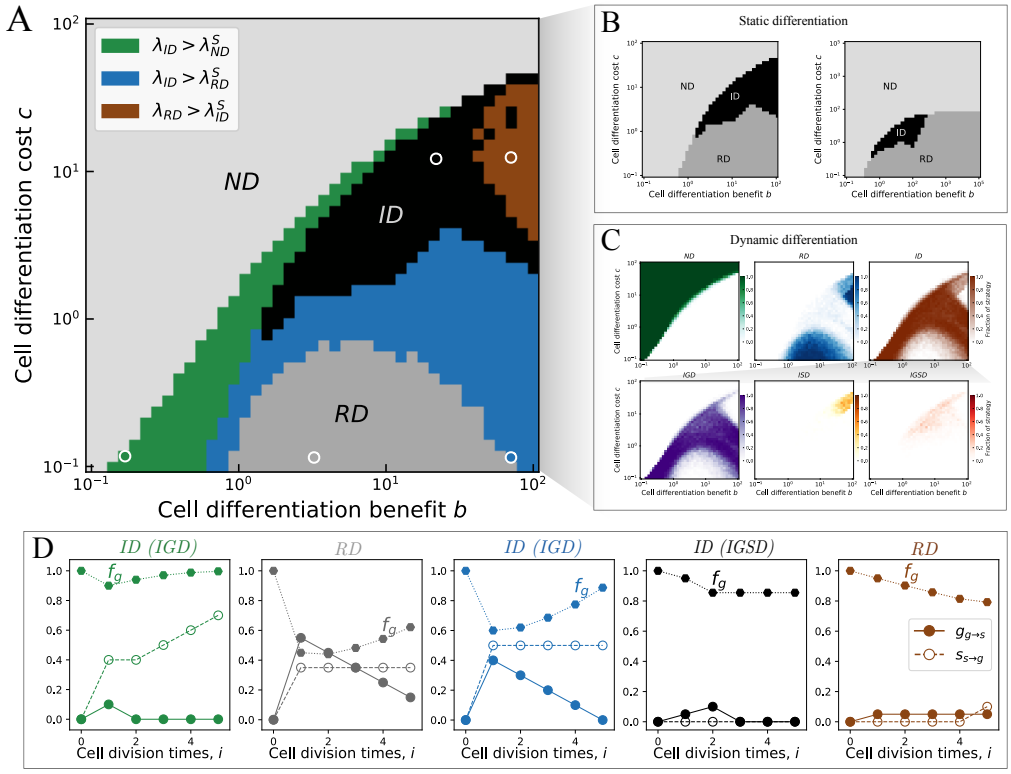


Fig 4. Dynamic differentiation strategies evolving under differentiation benefits and costs. A. Comparison of the parameter space of the optimal strategy between static (panel **B**) and dynamic cell differentiation (panel **C**). The grey, dark grey, and black areas represent the parameter space where the strategy ND, RD, and ID under static cell differentiation have the highest population growth rate λ. The green strip represents dynamic ID leading to a larger population growth rate than static ND. Similarly, the blue area and the brown area represent ID and RD leading to higher population growth rates than static RD and ID, respectively. **B.** Optimal static differentiation strategies at different scales of cell differentiation benefit and cost parameters. **C.** The frequencies of each dynamic strategy under cell differentiation benefits and costs. **D.** Cell differentiation probabilities ($g_{g\to s}$, $s_{s\to g}$) and the frequency of germ-like cell (f_g) via cell divisions at the parameter space indicated by white circles in panel **A**. The color is following that in panel **A**. Parameters of all panels: maximal cell division times $n = 5$, $0 \le \delta_i \le 0.1$, and $\alpha = \beta = 1$. At each pixel, the frequency of each optimal strategy was calculated across 100 replicates. Parameters of panels C: the number of initial sampling d_1, $M = 1000$, the number of dynamic strategies starting with a given d_1, $R = 100$, duplicates at each pixel is 100, for more detail, see S2 Appendix.

The optimal strategy turns into IGD again when b is much larger than c, but with higher soma-like transition probabilities $s_{s\to g}$ and higher fractions of germ-like cells, (the third panel of Fig 4D). Furthermore, analytical results show that except for $n = 1$ (S3 Appendix), either RD or ID is optimal in the absence of cell differentiation costs, i.e. $c = 0$ and $b > 0$ (S4 Appendix). Finally, we found that ID and RD lead to faster population growth than other strategies at high b and c. Specifically, a subcategory strategy $IGSD$ of ID evolves under small b, while a RD strategy evolves at large b, see the last two panels in Fig 4D. Increased differentiation benefits compensate for the effects of high differentiation costs of RD strategies on the population growth rate. Thus, RD with high cell transition probabilities is selected rather than $IGSD$.

In addition, we found irreversible cell differentiation ISD and $IGSD$ evolved for intermediate values of benefit and cost, see the lower right two panels in Fig 4C. We

found that both cell differentiation benefit and cost are indispensable factors for the evolution of ISD and $IGSD$, see the analytical proof in S5 Appendix. It is noteworthy that for static ISD, the only ID under static cell differentiation, the conclusion is consistent with our previous study [35]. At high differentiation benefit, we found that $IGSD$ is optimal at high c (the lower last two panels in Fig 4C). With ISD strategies, the transition from germ-like to soma-like cells still happens in the last cell division. However, with $IGSD$ strategies, no cell transition happens between the two types of cells in the last division. Therefore, compared with ISD strategies, $IGSD$ with both irreversible cell types bears lower cell differentiation costs. Meanwhile, it has relatively higher fractions of germ-like cells than $IGSD$, indicating a larger number of offspring, see Fig 2B. Thus, $IGSD$ can evolve at the condition of higher costs compared with ISD.

In addition, dynamic differentiation also promotes the evolution of irreversible cell differentiation under the effects of α and β [35], see S6 Appendix. We found that α plays a similar role under both dynamic and static differentiation. That is, irreversible differentiation strategies are optimal when $\alpha < 1$, i.e. the cell division rate component F_b accelerates with α, see Fig 2B and Fig 6. However, the effects of β, measuring the relative weight of cell transition between germ-like and soma-like cells, leads to different results between dynamic and static differentiation. We found that irreversible differentiation evolves across all values of β from small to large. As the subcategory IGD of ID evolves when β is small, and ISD and $IGSD$ of ID evolve when β is large. The effects of α and β are consistent with the conclusions of our previous study [35].

The maximum change of two successive differentiation probabilities determines the spectrum of population growth rates

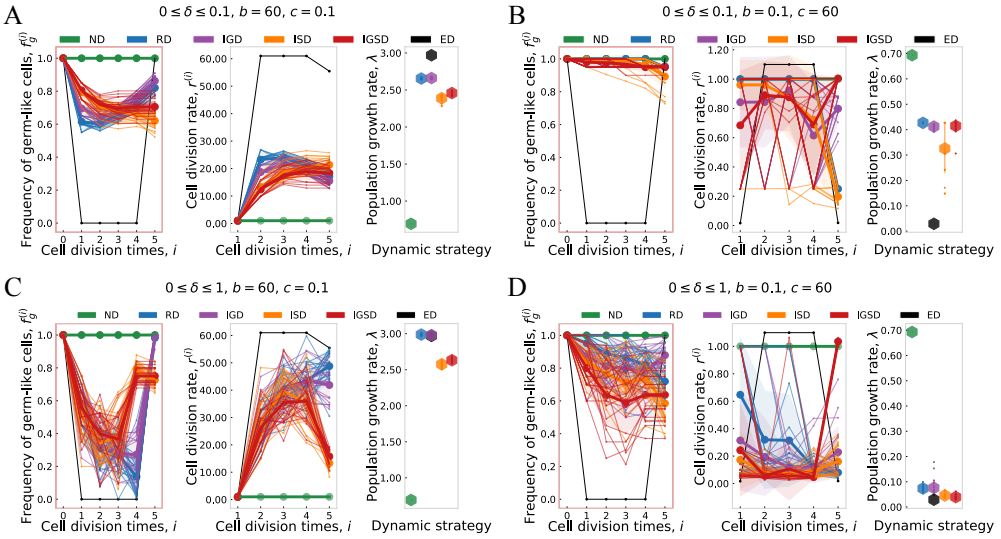


Fig 5. The effect of the maximum change of two successive differentiation probabilities δ_i on the population growth rate of optimal strategies. Frequencies of germ-like cells, cell division rates, and population growth rate of the optimal dynamic strategy of each category under $0 \le \delta_i \le 0.1$ (**A** and **B**) and under $0 \le \delta_i \le 1$ (**C** and **D**), $i = 1, 2, \ldots, n$. In all panels, the black diamond represents extreme differentiation ED. Thick lines are the averaged values at each cell division. The shaded areas indicate the standard deviation. $\alpha = \beta = 1$ and colors correspond with those in figure 2. Parameters: $n = 5$, and $\alpha = \beta = 1$, we chose 20 duplicates for generating the optimal strategies in each category.

Although arbitrary cell differentiation probabilities between consecutive cell divisions are not realistic, in theory, it is worth investigating the effects of the maximum change of two successive differentiation probabilities δ_i on the evolution of cell differentiation strategies, $i = 1, 2, \ldots, n$. We found that a higher maximum value of δ_i intensifies the effects of cell differentiation benefit and cost on the population growth rate, see Fig 5 A-D. When we change the maximum value of δ_i from 0.1 to 1, we found that population growth rates deviate more from the fixed population growth rate of ND, $\ln 2$. Population growth rate can be decomposed into cell division rates and the number of offspring (the fraction of germ-like cells after the nth division). The cell division rate is proportional to the fraction of soma-like cells but inversely proportional to the transition probability between two cell types. Taken together, the largest population growth rate favors the strategy with a higher fraction of soma-like cells all the time, a higher fraction of germ-like cells after the last cell division, and lower transition probabilities. As the sum of the fraction of germ-like cells and the fraction of soma-like cells is 1, the fractions of germ-like cells of optimal strategies show minima in the middle of our life cycle, especially when the benefit is large, see Fig 5. By contrast, the corresponding cell division rates have maxima for intermediate divisions in the optimal strategies when the benefits are large. The shape of the fraction of germ-like cells reveals how optimal strategies use the advantage of cell differentiation benefit to the maximum extent possible. When the maximum value of δ_i is higher, the fraction of germ-like cells and cell division rate across cell divisions change more substantially. Thus, a higher maximal value of δ_i provides amplification of the extent of the fractions of the germ-like cell and amplifies the effects of benefits and costs on the population growth rate. Specifically, when $0 \leq \delta \leq 0.1$, we found that IGD leads to the largest population growth rate, whereas when $0 \leq \delta \leq 1$, RD leads to the largest population growth rate, see Fig 5 A and C. IGD and RD lead to very close population growth rates under high benefits. Numerical results show that either IGD or RD are optimal under high benefits and RD is optimal under high costs, which is consistent with our analytical proof in the S3 Appendix and

Conclusion and discussion

We investigated the effect of dynamic differentiation on population growth and compared it with static cell differentiation. Static cell differentiation only allows a fixed cell differentiation probability for a cell type across cell divisions. Dynamic differentiation, by contrast, refers to being capable of changing differentiation probabilities in consecutive cell divisions. Dynamic differentiation intensifies the fluctuation of the germ-soma ratio during an organism's growth, which further increases the complexity of competition between different strategies. We used the population growth rate as a proxy to investigate the growth competition of different strategies under different benefits and costs. Based on the differentiation probabilities in the last division, we classify dynamic differentiation into three categories: non-differentiation ND, reversible differentiation RD, and irreversible differentiation ID. We found that dynamic differentiation favors ID (in the last division step) more than static differentiation in smaller organisms. Specifically, IGD leads to a higher population growth rate than ND and static reversible differentiation (static RD) in small organisms. ISD and $IGSD$ evolved in the parameter space with intermediate benefits and costs, consistent with previous findings [35]. In addition, the extent of dynamic variation in differentiation probability intensifies the effects of parameters on the population growth rate. The findings indicate that dynamic differentiation favors the evolution of irreversible cell differentiation, especially in small organisms.

That irreversible differentiation is favoured in small organisms is contrary to the

intuition provided by static differentiation, where irreversible differentiation is favoured as the organism size increases [35]. This discrepancy arises because of the emergence of stochastic developmental trajectories caused by dynamic differentiation. Under static differentiation, the developmental trajectories are very regular, while they can be much more complex in dynamic cell differentiation, see Fig 2B. These complex developmental trajectories in different categories increase the growth differences between different strategies. Thus, we found that the optimal strategies of differentiation categories can lead to divergent population growth rates. In addition, dynamic differentiation evolves two more subcategories than static one: irreversible germ differentiation IGD and irreversible germ and soma differentiation $IGSD$. The broad form of dynamic differentiation strategies can capture more cell differentiation patterns in reality. For example, the evolution of $IGSD$ can help us to understand cell lineage segregation in nature [38]. Our model can screen the stage where irreversible differentiation emerges, in line with the question of early segregation of germ and soma in animals [13, 39–42], but late in most plants [43].

Previous investigations of cell differentiation mostly focused on the state with a group of undifferentiated clonal cells [22, 24, 29–34, 44] or cells with randomly chosen initial cell types (similar to aggregated organisms) [34]. These models ignored the dynamic developmental trajectories of organisms from newborn to maturity. In our model, the developmental trajectories of each organism are recorded by dynamic differentiation probabilities, allowing us to know the dynamic fractions of each cell type during an organism's growth. Previously, Rodrigues et al. considered cell differentiation probability as an evolving trait in order to understand the evolution of differentiation [34]. They concluded that differentiation costs, compared with the difference in division rates between cell types, have less impact on the evolution of terminal and reversible differentiation. However, our model shows that differentiation benefits and costs play crucial roles in the evolution of differentiation strategies. When the cost is high, differentiation does not evolve. These inconsistent conclusions may be caused by the model assumptions in their model. They investigated developmental strategies in filament multicellular organisms with two essential tasks, but soma-like cells are not essential in our model.

While multicellularity can also form by aggregation [16, 45, 46], we assumed that organisms are clonal, growing from a single founding cell. The reasons for our clonal assumption are that multicellularity is formed commonly by clonal division rather than cell aggregation [16, 47], and clonal organisms with identical genes have advantages at purging deleterious mutations and reducing conflicts among cells [45, 48]. Therefore, clonal multicellularity is predicted to be evolutionarily stable [9]. In the cell differentiation models of aggregated multicellularity, a relatedness parameter can be used to evaluate the level of cooperation between cell types [27, 30, 31, 49].

We assumed that cell differentiation costs influence an organism's growth. In nature, cell differentiation and cell plasticity usually occur under severe environmental conditions, indicating a differentiation cost involved [10, 11, 50, 51]. Differentiation cost has been considered in previous theoretical research via varying forms [24, 27, 34, 52–54]. But the modeling purpose of cell differentiation costs is the same, i.e. reducing an organism's fitness. In our model, we are interested in the relative growth advantage between different differentiation strategies. Therefore, we assume that differentiation costs affect the population growth rate, reducing cell division rates. Finally, we suppose that cells undergo synchronous cell divisions. This is not true for large multicellularity with many more cell divisions [38, 55]. Asynchronous cell division has been explored under static differentiation in previous studies, leading to the same predictions as the synchronous one [35]. Yet, we still need to investigate whether asynchronous cell division leads to the same conclusion as synchronous ones under dynamic differentiation

in the future. Our model could be further extended by including cell death or differentiation costs related to risk of organism death. Yet, our model gives first insights into understanding the effects of dynamic differentiation on the evolution of cell differentiation in multicellularity.

Supporting information

S1 Appendix. Population growth rate. In our model, we treat both the number of cells and growth time as continuous, distilling the stochastic process down to two quantities for calculating the population growth rate: the expected offspring number of germ-like cells N and the amount of growth time for an organism to grow t. The population growth rate λ can be calculated by the following equation

$$\lambda = \frac{\ln N}{t}. \tag{9}$$

We use $f_g(i)$ and $f_s(i)$ to denote the fractions of germ-like cell and soma-like cell after the ith cell division. Since each organism starts with a single germ-like cell, $f_g(0) = 1$ and $f_s(0) = 0$. We use $p^{(i)}_{x\to y}$ to denote the transition probability from cell type x to y in the ith cell division, where x and y are either germ-like cells or soma-like cells. Based on Eq (1), we have

$$\begin{aligned} g^{(i)}_{g\to g} &= g^{(i)}_{gg} + \frac{g^{(i)}_{gs}}{2} \\ g^{(i)}_{g\to s} &= g^{(i)}_{ss} + \frac{g^{(i)}_{gs}}{2} \\ s^{(i)}_{s\to g} &= s^{(i)}_{gg} + \frac{s^{(i)}_{gs}}{2} \\ s^{(i)}_{s\to s} &= s^{(i)}_{ss} + \frac{s^{(i)}_{gs}}{2}. \end{aligned} \tag{10}$$

After the ith cell division, the expected fraction of germ-like cells is $f^{(i)}_g = g^{(i)}_{g\to g} f^{(i-1)}_g + s^{(i)}_{s\to g} f^{(i-1)}_s$ and the expected fraction for soma-like cells is $f^{(i)}_s = g^{(i)}_{g\to s} f^{(i-1)}_g + s^{(i)}_{s\to s} f^{(i-1)}_s$, which can be expressed in

$$\begin{pmatrix} f^{(i)}_g \\ f^{(i)}_s \end{pmatrix} = \begin{pmatrix} g^{(i)}_{g\to g} & s^{(i)}_{s\to g} \\ g^{(i)}_{g\to s} & s^{(i)}_{s\to s} \end{pmatrix} \begin{pmatrix} f^{(i-1)}_g \\ f^{(i-1)}_s \end{pmatrix}. \tag{11}$$

The expected $f^{(n)}_g$ and $f^{(n)}_s$ can be calculated recursively by Eq (11)

$$\begin{pmatrix} f^{(n)}_g \\ f^{(n)}_s \end{pmatrix} = \begin{pmatrix} g^{(n)}_{g\to g} & s^{(n)}_{s\to g} \\ g^{(n)}_{g\to s} & s^{(n)}_{s\to s} \end{pmatrix} \cdots \begin{pmatrix} g^{(i)}_{g\to g} & s^{(i)}_{s\to g} \\ g^{(i)}_{g\to s} & s^{(i)}_{s\to s} \end{pmatrix} \cdots \begin{pmatrix} g^{(1)}_{g\to g} & s^{(1)}_{s\to g} \\ g^{(1)}_{g\to s} & s^{(1)}_{s\to s} \end{pmatrix} \begin{pmatrix} f^{(0)}_g \\ f^{(0)}_s \end{pmatrix}. \tag{12}$$

Since cells divide synchronously and no cell dies during growth, the expected number of germ-like cells $N^{(n)}_g$ and soma-like cells $N^{(n)}_s$ after the nth cell division are

$$\begin{pmatrix} N^{(n)}_g \\ N^{(n)}_s \end{pmatrix} = 2^n \begin{pmatrix} f^{(n)}_g \\ f^{(n)}_s \end{pmatrix}, \tag{13}$$

where $0 \le f^{(n)}_g, f^{(n)}_s \le 1$.

The cell division rate determines the growth duration of organisms. Since cells divide with a rate $r^{(i)} = \frac{1+b[f_s^{(i-1)}]^\alpha}{1+c[f_{g\to s}^{(i)}+\beta f_{s\to g}^{(i)}]}$ during the ith cell division, the waiting time for a cell division $t^{(i)}$ follows the exponential distribution $f(t^{(i)}) = r^{(i)}e^{-r^{(i)}t^{(i)}}$, where $f_{g\to s}^{(i)} = f_g^{(i-1)} s_{s\to g}^{(i)}$ and $f_{s\to g}^{(i)} = f_s^{(i-1)} s_{s\to g}^{(i)}$, see Eq (6). Thus the expected waiting time from the ith cell division to the $(i+1)$th cell division is $t^{(i)} = \frac{1}{r^{(i)}}$. The expected growth time for organisms with total n cell divisions is

$$t = \sum_{i=1}^{n} t^{(i)} = \sum_{i=1}^{n} \frac{1}{r^{(i)}} = \sum_{i=1}^{n} \frac{1 + c[f_g^{(i-1)} g_{g\to s}^{(i)} + \beta f_s^{(i-1)} s_{s\to g}^{(i)}]}{1 + b[f_s^{(i-1)}]^\alpha}. \tag{14}$$

Substituting Eq (13) and Eq (14) into Eq (9), we have

$$\lambda = \frac{\ln N}{t} = \frac{n \ln 2 + \ln f_g^{(n)}}{\sum_{i=1}^{n} \frac{1+c\left[f_g^{(i-1)} g_{g\to s}^{(i)} + \beta f_s^{(i-1)} s_{s\to g}^{(i)}\right]}{1+b[f_s^{(i-1)}]^\alpha}}, \tag{15}$$

where n is the number of total cell divisions of organisms, $f_g^{(i)}$ and $f_s^{(i)}$ are fractions of germ-like cell and soma-like cell after the ith cell division, $g_{g\to s}^{(i)}$ and $s_{s\to g}^{(i)}$ are the transition probabilities between germ-like cell and soma-like cell at the ith cell division $(1 \le i \le n)$. We have $f_g^{(0)} = 1$ and $f_s^{(0)} = 0$. For the non-differentiation strategy ND, no soma-like cells are produced during growth, i.e. $g_{g\to g} = 1$ and $g_{g\to s} = s_{s\to g} = s_{s\to s} = 0$. Therefore, $f_g^{(i)} = 1$, $f_s^{(i)} = 0$. Thus from Eq (15) the population growth rate of ND which is denoted by λ_{ND} is $\ln 2$. Biologically, the population growth rate of ND describes the number of cells doublings per unit of time.

S2 Appendix. Numerical calculation of the population growth rate of the dynamic differentiation strategy. We introduce the method of calculating population growth rate numerically in dynamic cell differentiation. We first look at the cell differentiation probabilities in the first division step $d_1 = [g_{gg}^{(1)}, g_{gs}^{(1)}, g_{ss}^{(1)}, s_{gg}^{(1)}, s_{gs}^{(1)}, s_{ss}^{(1)}]$. Each probability can take the value 0 or other values by increasing 0.1 from 0 at each time until reaching the highest value 1, thus there are 11 possible values for each probability, i.e $0, 0.1, 0.2, \ldots, 1$. We first define the number of probability combinations. Since $g_{gg}^{(1)} + g_{gs}^{(1)} + g_{ss}^{(1)} = 1$ and $g_{ss}^{(1)} = 1 - g_{gg}^{(1)} + g_{gs}^{(1)}$, as long as we know the values of $g_{gg}^{(1)}$ and $g_{gs}^{(1)}$, we know g_{ss}. When $g_{gg} = 0$, g_{gs} can take the 11 values from 0 to 1, Thus, there are totally $\sum_{i=1}^{11} i = 66$ combinations for $g_{gg}^{(1)}, g_{gs}^{(1)}, g_{ss}^{(1)}$. The same number of combinations exist for soma-like cells. Thus, there are a total of $66 \times 66 = 4356$ combinations for d_1. As long as d_1 is chosen, we need to identify d_2, $d_3, \ldots, d_n$. d_2 deviates from d_1 by either a 0 or δ_2. That is $0 \le |g_{gg}^{(1)} - g_{gg}^{(2)}| \le \delta_2$. The same for the other probabilities $g_{gs}, g_{ss}, s_{gg}, s_{gs}, s_{ss}$. The choice of d_{i+1} depends on number of neighbours of d_i, which further depends on the elements d_i. For $\delta_{i+1} = 0.1$, if $g_{gg} = s_{ss} = 1$ in d_i, then g_{gg} and s_{ss} can only be decreased or be constant. Thus, there are 5 choices for d_{i+1}. However, if the elements in d_i are either 0.3 or 0.4, then each element can be increased, decreased, or unchanged. Therefore, it has 13 choices for choosing d_{i+1}. Let's take $d_1 = [0.3, 0.3, 0.4, 0.3, 0.3, 0.4]$ as an example, then it's

neighbours are

$$
\begin{aligned}
&[0.3, 0.3, 0.4, 0.3, 0.3, 0.4],\\
&[0.4, 0.2, 0.4, 0.3, 0.3, 0.4],\\
&[0.4, 0.3, 0.3, 0.3, 0.3, 0.4],\\
&[0.2, 0.4, 0.4, 0.3, 0.3, 0.4],\\
&[0.2, 0.3, 0.5, 0.3, 0.3, 0.4],\\
&[0.3, 0.4, 0.3, 0.3, 0.3, 0.4],\\
&[0.3, 0.2, 0.5, 0.3, 0.3, 0.4],\\
&[0.3, 0.3, 0.4, 0.4, 0.2, 0.4],\\
&[0.3, 0.3, 0.4, 0.4, 0.3, 0.3],\\
&[0.3, 0.3, 0.4, 0.2, 0.4, 0.4],\\
&[0.3, 0.3, 0.4, 0.2, 0.3, 0.5],\\
&[0.3, 0.4, 0.3, 0.3, 0.3, 0.4],\\
&[0.3, 0.3, 0.4, 0.3, 0.2, 0.5].
\end{aligned}
$$

Note that d_1 is considered as one of its own neighbours. To generate a dynamic differentiation strategy, we first chose d_1 from the combination pool and then chose d_2 from d_1's neighbors and repeat the process until obtaining d_n.

In static cell differentiation, we calculate the population growth rates of each strategy in the cell differentiation probabilities pool. We seek the optimal strategy which leads to the fastest growing population among these 4356 strategies. To find the optimal strategy at a given parameter point, we first chose $M = 1000$ values for d_1 from the cell differentiation pool. Then for each chosen d_1, we randomly chose $R = 100$ dynamic strategies, all generated from this d_1. $R = 100$ is the sampling size of the dynamic strategies from the same initial differentiation probabilities d_1. Then we compute the population growth rate of the 10^5 strategies and choose the strategy leading to the largest growth rate. Next, we optimize that strategy further. For the optimal strategy with the largest growth rate, we compare its growth rate with a slightly modified strategy. The modified strategies include the one removing d_1 but compensating with an d_{n+1} or removing d_n by compensating with an d_0. Specifically, for the focused $D = [d_1, d_2, \ldots, d_n]$, we check whether $D' = [d_0, d_1, d_2, \ldots, d_{n-1}]$ or $D' = [d_2, \ldots, d_n, d_{n+1}]$ leads to a higher growth rate over D. Here the d_0 is one neighbour of d_1, and d_{n+1} is one neighbour of d_n. If the D' leads to a higher population growth rate, we keep the process until finding the D' which makes the population growth rate stays at the maxima. We aim to find a local optimum close to the strategy that was identified in our grid search. Local optimization stops when the largest steady growth rate in the local neighbourhood is identified. Overall, we first search the optimal D globally by randomly choosing d_1, represented by M and R. Then, we used a local grid search by modifying d_1 or d_n of a strategy until finding the optimal D. Besides, we have constructed initial sampling strategies from the middle of d_i sequences. We first identified $d_{\frac{n+1}{2}}$ if n is odd and $d_{\frac{n}{2}}$ if n is even, and then constructed the rest d_is. The results show that there is almost no differences in terms of searching for optimal strategies between the two methods.

S3 Appendix. Optimality of non-differentiation strategy ND.

ND is optimal in the absence of cell differentiation benefits for any maximal cell division number n

When the cell differentiation benefit is absent, i.e. $b = 0$ and $c > 0$, we find that ND is the optimal strategy based on Eq (15).

ND is optimal in the absence of costs for $n = 1$

Next, when there is only one cell division ($n = 1$), we prove that ND leads to the largest population growth rate under $b > 0$ and $c = 0$. Since $f_g^{(0)} = 1$ and $f_s^{(0)} = 0$, based on Eq (14), the growth time is

$$t = \sum_{i=1}^{n=1} t^{(i)} = \frac{1}{r^{(1)}} = \frac{1}{1 + b[f_s^{(0)}]^\alpha} = 1. \tag{16}$$

Substituting Eq (16) into Eq (15) and using Eq (12), we have

$$\lambda = \frac{\ln N}{t} = \ln 2 + \ln f_g^{(1)} = \ln 2 + \ln g_{g\to g}^{(1)}. \tag{17}$$

Since $0 \leq g_{g\to g}^{(1)} \leq 1$, the optimal strategy is ND which has $g_{g\to g}^{(1)} = 1$. Thus, ND is the optimal strategy under $b > 0$, $c = 0$ and $n = 1$.

S4 Appendix. Either IGD or RD is optimal in the absence of cell differentiation costs when maximal cell division $n > 1$. To show the optimal strategy is either IGD or RD under $b > 0$ and $c = 0$. We first prove $\lambda_{IGD} > \lambda_{IGSD} > \lambda_{ND}$, and then prove $\lambda_{RD} > \lambda_{ISD} > \lambda_{ND}$.

IGD is optimal among IGD, $IGSD$ and ND

To prove $\lambda_{IGD} > \lambda_{IGSD} > \lambda_{ND}$, we begin with the proof of $\lambda_{IGSD} > \lambda_{ND}$. Unlike the ND strategy, ISD, RD, IGD and $IGSD$ are categories which include many strategies. As long as one strategy in $IGSD$ has a greater population growth rate than ND, we say $IGSD$ is more optimal than ND. Think of an $IGSD$ strategy with only a non-zero cell differentiation probability from germ-like cells to soma-like cells and zero differentiation probabilities the other way around. Let's assume it is the ith cell division that makes $g_{g\to s}^{(i)} > 0$, thus we have $g_{g\to s}^{(j)} = 0$ for $j \neq i$ and $s_{s\to g}^{(i)} = 0$ for any i. Based on Eq (12), the cell frequencies after the nth division are

$$\begin{aligned}\begin{pmatrix} f_g^{(n)} \\ f_s^{(n)} \end{pmatrix} &= \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \cdots \begin{pmatrix} g_{g\to g}^{(i)} & 0 \\ g_{g\to s}^{(i)} & 1 \end{pmatrix} \cdots \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \begin{pmatrix} f_g^{(0)} \\ f_s^{(0)} \end{pmatrix} \\ &= \begin{pmatrix} g_{g\to g}^{(i)} \\ g_{g\to s}^{(i)} \end{pmatrix},\end{aligned} \tag{18}$$

where $f_g^{(0)} = 1$ and $f_s^{(0)} = 0$. For convenience, we denote $g_{g\to g}^{(i)} = g^*$ and $g_{g\to s}^{(i)} = 1 - g^*$. From Eq (15), we have

$$\begin{aligned}
\lambda_{IGSD} &= n \ln 2 + \ln g^* \frac{n-1}{1+b} + \frac{1}{1+b(1-g^*)^\alpha} \\
&\geq \frac{n \ln 2 + \ln g^*}{\frac{n+b}{1+b}} \\
&= \ln 2 + \frac{(n-1)b}{n+b} \ln 2 + \frac{1+b}{n+b} \ln g^* \\
&= \lambda_{ND} + \frac{\ln 2^{(n-1)b} (g^*)^{1+b}}{n+b},
\end{aligned} \tag{19}$$

where we use $(1 - g^*) \geq 0$ to obtain the inequality and $\lambda_{ND} = \ln 2$ (appendix). Since $b > 0$ and $n > 1$, as long as $2^{(n-1)b}(g^*)^{1+b} \geq 1$, we obtain $\lambda_{IGSD} > \lambda_{ND}$. That is $g^* \geq 12^{\frac{(n-1)b}{1+b}}$. Therefore, when $b > 0$, we can always find an $IGSD$ strategy with a $g_{g\to s}^{(i)} \leq 1 - 12^{\frac{(n-1)b}{1+b}}$ and all other $g_{g\to s}^{(i)} = 0$ and $s_{s\to g}^{(i)} = 0$, which leads to higher population growth rate than ND. Thus, $\lambda_{IGSD} > \lambda_{ND}$. The proof of $\lambda_{IGD} > \lambda_{IGSD}$ is in the appendix . Taken these together, we have $\lambda_{IGD} > \lambda_{IGSD} > \lambda_{ND}$.

RD is optimal among RD, ISD and ND

Next, we prove $\lambda_{RD} > \lambda_{ISD} > \lambda_{ND}$. We first prove $\lambda_{ISD} > \lambda_{ND}$. We prove that there exists an ISD strategy leading to a higher λ than $\lambda_{ND} = \ln 2$ (appendix). Consider the ISD with $s_{s\to s}^{(i)} = 1$, but with at least one i which makes $g_{g\to s}^{(i)} > 0$ i.e. $g_{g\to g}^{(i)} = 1 - g_{g\to s}^{(i)} < 1$ for $1 \leq i \leq n$. The above constraint corresponds with the definition of the ISD strategy. Based on Eq (12), the cell frequencies after the nth division are

$$\begin{aligned}
\begin{pmatrix} f_g^{(n)} \\ f_s^{(n)} \end{pmatrix} &= \begin{pmatrix} g_{g\to g}^{(n)} & 0 \\ g_{g\to s}^{(n)} & 1 \end{pmatrix} \cdots \begin{pmatrix} g_{g\to g}^{(i)} & 0 \\ g_{g\to s}^{(i)} & 1 \end{pmatrix} \cdots \begin{pmatrix} g_{g\to g}^{(1)} & 0 \\ g_{g\to s}^{(1)} & 1 \end{pmatrix} \begin{pmatrix} f_g^{(0)} \\ f_s^{(0)} \end{pmatrix} \\
&= \begin{pmatrix} \prod_{i=1}^n g_{g\to g}^{(i)} \\ 1 - \prod_{i=1}^n g_{g\to g}^{(i)} \end{pmatrix},
\end{aligned} \tag{20}$$

where $n \geq 1$. Substituting Eq (20) into Eq (15) and together with $c = 0$, we find the growth rate of the ISD strategy

$$\lambda_{ISD} = n \ln 2 + \ln \prod_{i=1}^n g_{g\to g}^{(i)} 1 + \sum\nolimits_{i=2}^n \frac{1}{1 + b[1 - \prod_{k=1}^{i-1} g_{g\to g}^{(k)}]^\alpha}, \tag{21}$$

where the first item in the denominator represents the time for the first cell division $t_1 = 1$ because of $f_s^{(0)} = 0$. We define the second item of the denominator of Eq (21) as $F(n) = \sum_{i=2}^n f^{(i)}$, where $f^{(i)} = 11 + b[1 - \prod_{k=1}^{i-1} g_{g\to g}^{(k)}]^\alpha$. Next, we prove $F(n)$ is a bounded function. Since $0 \leq g_{g\to g}^{(k)} \leq 1$, thus sequence $\{\prod_{k=1}^{i-1} g_{g\to g}^{(k)}\}_{i=2}^\infty$ decreases with increasing i. That is, $\{f^{(i)}\}_{i=2}^\infty$ is a positive but decreasing sequence. $f^{(2)}$ is the largest one in $\{f^{(i)}\}_{i=2}^\infty$. Therefore, the sequence $\{F(n) = \sum_{i=2}^n f^{(i)}\}$ is an accelerating discrete sequence with respect to n. We have

$$F(n) \leq (n-1) f^{(2)} = \frac{n-1}{1 + b\left(1 - g_{g\to g}^{(1)}\right)^\alpha}. \tag{22}$$

Substituting the right-hand side of inequality (22) into Eq (21), we have

$$\begin{aligned}
\lambda_{ISD} &= n\ln 2 + \ln\prod_{i=1}^{n} g_{g\to g}^{(i)} \sum\nolimits_{i=1}^{n} \frac{1}{1+b(1-\prod_{i=1}^{n} g_{g\to g}^{(i)})^{\alpha}} \\
&\geq n\ln 2 + \ln\prod_{i=1}^{n} g_{g\to g}^{(i)} 1 + \frac{n-1}{1+b\left(1-g_{g\to g}^{(1)}\right)^{\alpha}} \\
&= \left(n\ln 2 + \ln\prod_{i=1}^{n} g_{g\to g}^{(i)}\right)[1+b\left(1-g_{g\to g}^{(1)}\right)^{\alpha}]n + b\left(1-g_{g\to g}^{(1)}\right)^{\alpha} \\
&= \ln 2 + (n-1)b\left(1-g_{g\to g}^{(1)}\right)^{\alpha}\ln 2 + [1+b\left(1-g_{g\to g}^{(1)}\right)^{\alpha})]\ln\prod_{i=1}^{n} g_{g\to g}^{(i)} n + b\left(1-g_{g\to g}^{(1)}\right)^{\alpha}.
\end{aligned} \tag{23}$$

As long as there exist a strategy which makes the right side of Eq (23) greater than $\ln 2$, we have $\lambda_{ND} < \lambda_{ISD}$. Then we need to identify the conditions for

$$(n-1)b\left(1-g_{g\to g}^{(1)}\right)^{\alpha}\ln 2 + (1+b\left(1-g_{g\to g}^{(1)}\right)^{\alpha})\ln\prod_{i=1}^{n} g_{g\to g}^{(i)} > 0 \tag{24}$$

to hold. As $b > 0$ and $\alpha > 0$, $(n-1)b\left(1-g_{g\to g}^{(1)}\right)^{\alpha}\ln 2 \geq 0$. Since $0 \leq g_{g\to g}^{(i)} \leq 1$, $\prod_{i=1}^{n} g_{g\to g}^{(i)} \leq 1$ and $\ln\prod_{i=1}^{n} g_{g\to g}^{(i)} \leq 0$. The second item of Eq (24) is negative. There exists a sequence $\{g_{g\to g}^{(i)}\}$, which makes $\prod_{i=1}^{n} g_{g\to g}^{(i)} \to 1^{-}$ and

$$\begin{aligned}
\ln\prod_{i=1}^{n} g_{g\to g}^{(i)} &> -(n-1)b\left(1-g_{g\to g}^{(1)}\right)^{\alpha}\ln 21 + b\left(1-g_{g\to g}^{(1)}\right)^{\alpha} \\
&= -(n-1)\ln 21 + \frac{1}{b\left(1-g_{g\to g}^{(1)}\right)^{\alpha}},
\end{aligned} \tag{25}$$

which makes the Eq (24) hold. With the above proof, we conclude that $\lambda_{ISD} > \lambda_{ND}$ with only cell differentiation benefit. From Eq (25), we found that more ISD strategies are better than ND under high benefits b. However, when b is small, only ISD with $g_{g\to g}^{(i)} \to 1$ leads higher population growth rate than ND. The proof of $\lambda_{RD} > \lambda_{ISD}$ can be found in the appendix . Thus, we have $\lambda_{RD} > \lambda_{ISD} > \lambda_{ND}$. The results show that when there is a benefit and no costs, differentiation strategies (ISD, $IGSD$, IGD and RD) are better over ND. Either IGD or RD is optimal under $b > 0$ and $c = 0$.

S5 Appendix. $IGSD$ and ISD cannot be optimal in the absence of either cell differentiation benefit or cost. In the appendix , we have proved ND is optimal in the absence of differentiation benefits, i.e. $b = 0$ and $c > 0$. Thus, we prove $IGSD$ and ISD can be optimal in the absence of differentiation costs, i.e. $c = 0$ and $b > 0$. Since we also have proved that ND is optimal under $n = 1$ when $c = 0$ and $b > 0$ in appendix . Therefore, we only need to prove that the optimal strategy can neither be $IGSD$ nor ISD when $b > 0$, $c = 0$ and $n \geq 2$.

We first prove $\lambda_{IGD} > \lambda_{IGSD}$. For a given $IGSD$ strategy, we can always modify it and obtain an IGD strategy, which leads to a higher λ than the given $IGSD$ strategy. For a given $IGSD$ strategy, we know its transition probabilities $s_{s\to g}^{(n)} = 0$. We modify the $IGSD$ strategy by setting $0 < s_{s\to g}^{(n)} = k \leq 1$ to get a IGD strategy. The constructed IGD strategy produces more offspring than the given $IGSD$ strategy as its

final number of germ-like cells is $N = 2^n(f_g^{(n-1)} g_{g\to g}^{(n)} + f_s^{(n-1)} s_{s\to g}^{(n)})$, which is greater than that of the $IGSD$ as $s_{s\to g}^{(n)} = k > 0$ in the IGD strategy. Since there is no cell differentiation cost ($c = 0$), cell division rates are the same among all strategies. Thus, $\lambda_{IGD} > \lambda_{IGSD}$.

Next, we prove $\lambda_{RD} > \lambda_{ISD}$. Given an ISD strategy, we have $s_{s\to g}^{(n)} = 0$. Construct a RD strategy that has the same transition probability matrixes as the given ISD strategy for the first $(n-1)$ cell divisions. For the nth transition probability matrix, we keep the $g_{g\to g}^{(n)}$ and $g_{g\to s}^{(n)}$ the same as that in the given ISD strategy. However, we set $s_{s\to g}^{(n)} > 0$ rather than $s_{s\to g}^{(n)} = 0$ as that in ISD strategy. $s_{s\to g}^{(n)} > 0$ implies $s_{s\to s}^{(n)} = 1 - s_{s\to g}^{(n)} < 1$. Then, ISD and RD have the same germ-like cells during the first $n-1$ cell divisions. The fraction of germ-like cells for the ISD strategy after the nth cell divisions is $f_g^{(n-1)} g_{g\to g}^{(n)} + f_s^{(n-1)} s_{s\to g}^{(n)} = f_g^{(n-1)} g_{g\to g}^{(n)}$ as $s_{s\to g}^{(n)} = 0$. Whereas, the fraction of germ-like cells for the RD strategy after the nth cell divisions is $f_g^{(n-1)} g_{g\to g}^{(n)} + f_s^{(n-1)} s_{s\to g}^{(n)}$. Thus, the constructed RD has an extra $2^n f_s^{(n-1)} s_{s\to g}^{(n)}$ germ-like cells compared with the ISD strategy. Since the cell division rate at the nth cell division depends on the fraction of soma-like cells at the $(n-1)$th cell division, thus the cell division rates $r^{(i)}$ for the two strategies are the same, $1 \le i \le n$. Thus, from Eq (15), we have

$$\begin{aligned}
\lambda_{RD} &= \ln\{2^n[f_g^{(n-1)} g_{g\to g}^{(n)} + f_s^{(n-1)} s_{s\to g}^{(n)}]\} \sum_{i=1}^{n} \frac{1}{1 + b[f_s^{(i-1)}]^\alpha} \\
&> \ln\{2^n[f_g^{(n-1)} g_{g\to g}^{(n)}]\} \sum_{i=1}^{n} \frac{1}{1 + b[f_s^{(i-1)}]^\alpha} \\
&= \lambda_{ISD}.
\end{aligned} \tag{26}$$

Therefore, $\lambda_{RD} > \lambda_{ISD}$.

S6 Appendix. Dynamic differentiation promotes irreversible cell differentiation under the effects of benefit function forms α and the ratio of differentiation costs between germ-like cells and soma-like cells β.

Under the effects of α and β, we found that dynamic differentiation favors irreversible cell differentiation over static cell differentiation. IGD replaces static RD when α and β are both small, see Fig 6. Under this scenario, the cell transition probability $s_{s\to g}$ has a smaller effect in decreasing the population growth rate than the transition probability $g_{g\to s}$. Thus, IGD produces a higher fraction of germ-like cells and bears less cell differentiation costs, leading to a higher population growth rate. When α is around 1, IGD leads to faster growth than ND. The reason is analogous to the one given in the main text.

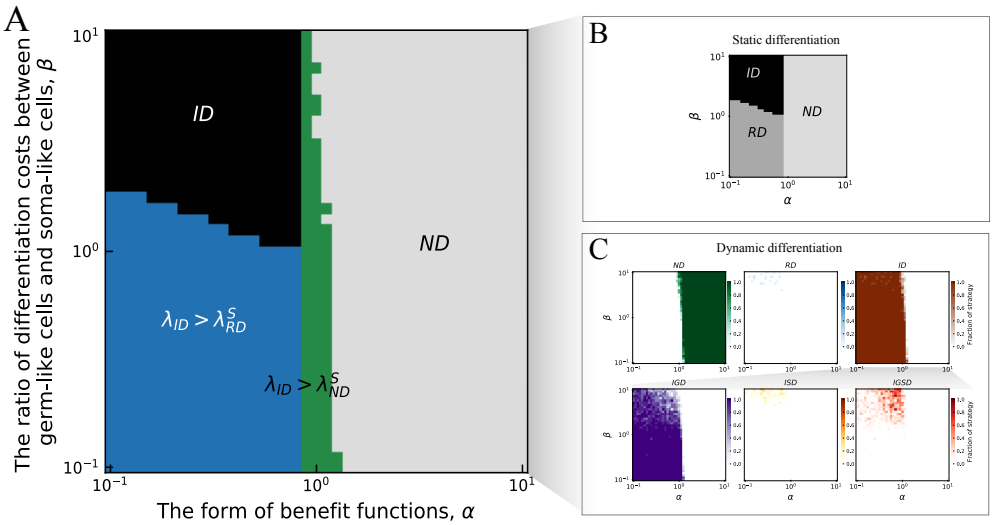


Fig 6. The effects of α and β on the population growth rates of cell differentiation strategies. A. Comparison of the optimal strategy evolved in static and dynamic differentiation strategies depending on α and β. The areas of grey and black represent the parameter space in which strategy ND and ID are optimal both under static and dynamic cell differentiation. The green area represents dynamic ID leading to a larger population growth rate than static ND. The blue strip represents dynamic ID leading to a larger population growth rate than static RD. **B.** The parameter space of optimal static differentiation strategy at different values of α and β. **C.** The frequencies of each dynamic strategy depending on α and β. Parameters for all panels $\delta = 0.1$, $n = 5$ and $b = c = 1$. For calculating the population growth rate of each strategy, see the appendix .

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