The possible modes of microbial reproduction are fundamentally restricted by distribution of mass between parent and offspring

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Multiple modes of asexual reproduction are observed among microbial organisms in natural populations. These modes are not only subject to evolution, but may drive evolutionary competition directly through their impact on population growth rates. The most prominent transition between two such modes is the one from unicellularity to multicellularity. We present a model of the evolution of reproduction modes, where a parent organism fragments into smaller parts. While the size of an organism at fragmentation, the number of offspring, and their sizes may vary a lot, the combined mass of fragments is limited by the mass of the parent organism. We found that mass conservation can fundamentally limit the number of possible reproduction modes. This has important direct implications for microbial life: For unicellular species, the interplay between cell shape and kinetics of the cell growth implies that the largest and the smallest possible cells should be rod shaped rather than spherical. For primitive multicellular species, these considerations can explain why rosette cell colonies evolved a mechanistically complex binary split reproduction. Finally, we show that the loss of organism mass during sporulation can explain the macroscopic sizes of the formally unicellular microorganism *Myxomycetes* plasmodium. Our findings demonstrate that a number of seemingly unconnected phenomena observed in unrelated species may be different manifestations of the same underlying process.

reproduction | growth competition | mass conservation

The ability of living beings to reproduce is an essential ingredient for natural selection to operate (1, 2), and often included in definitions of life itself (3). Across the life forms present on Earth, there is a great variety in reproduction modes. Even in the simplest case of asexual reproduction in microorganisms, there is huge diversity, for example, production of small propagules, the fragmentation of a body into two equal parts and as a fission into multiple pieces (4–9). This observation immediately leads to a number of questions: Why is such a diversity observed? What are the selective forces driving the evolution of microbial reproduction modes? What kind of reproduction modes can we expect to observe among microbial species still waiting to be discovered?

These topics are addressed by life history theory, which was originally developed for macroscopic organisms (10–15), such as animals and higher plants. Life cycles of microscopic species, however, evolve under a very different set of conditions. For example, they have extremely limited perception and evaluation abilities, and, as a consequence, their behavior is a scripted choice from a limited number of strategies rather than any form of decision-making. The reproductive time scale of microscopic species is incredibly fast compared to environmental processes—multiple microbial generations may pass within a single day. Therefore, the stochasticity of the environment (12, 16, 17) is a factor that tends to be of relatively minor importance compared to macroscopic species. Typically, the cell division (or colony fragmentation) is essentially semelparous: No unambiguous "parent" individual remains—all resulting organisms are "offspring." Moreover, mass conservation is crucial on the level of microbial life. For example, the 16-cell algae *Gonium pectorale* reproduces by fission of the connections between cells. The result of such a fragmentation is 16 independent cells (18) and not a colony. Each of the parts is smaller than the parental organism, and they have to regrow before being able to reproduce again. Finally, the fluid structure of the cell allows for an incredible diversity of possible reproduction modes. And, indeed, microscopic beings demonstrate an astonishing number of ways of producing offspring. Often, these reproduction modes are simply taken for granted, and the overall understanding of what can drive evolution of microscopic life cycles is, so far, lacking.

From another perspective, the growth of microscopic organisms is actively studied in biophysics (19–23). Yet, these models focus on the laws of cell growth rather than patterns of cell division. For example, recently, a mechanistic model of *Escherichia coli* cell division was proposed (24). There, the authors suggest the mechanism of division licensing, which ensures the execution of particular patterns of cell division. Nevertheless, the question of

Significance

Cells and simple cell colonies reproduce by fragmenting their bodies into pieces. Produced newborns need to grow before they can reproduce again. How big a cell or a cell colony should grow? How many offspring should be produced? Should they be of equal size or diverse? We show that the simple fact that the immediate mass of offspring cannot exceed the mass of parents restricts possible answers to these questions. For example, our theory states that, when mass is conserved in the course of fragmentation, the evolutionarily optimal reproduction mode is fragmentation into exactly two, typically equal, parts. Our theory also shows conditions which promote evolution of asymmetric division or fragmentation into multiple pieces.

why the division of *E. coli* into two parts as we observe it has an adaptive advantage, in the first place, remains open.

A similar discussion arises in the context of the evolution of multicellularity, which, recently, has gotten a lot of attention from experimentalists (25–28) and theoreticians (29–39). Yet, the question of the evolution of reproduction modes is broader than the evolution of multicellularity. One of the major drawbacks of models for the evolution of multicellularity is the assumption of the discreteness—colonies are composed of cells which do not change in time, and cells are born already identical to any other cell in a population (34–38, 40). This assumption does not reflect the gradual accumulation of mass in the course of the organism growth (41). These models are thus not good descriptions of scenarios in which cells comprising the offspring differ in size from cells forming the parent (42–44).

In this work, we developed a model of the population dynamics of continuously growing organisms reproducing by fragmentation into smaller parts. In our model, the rate of growth of an individual organism depends on its mass. Hence, populations employing different reproduction modes—the maturity mass at which the fragmentation occurs, the number of offspring, and their masses—will have different population growth rates. Using this model, we analyzed the evolutionary competition between such populations by comparing their population growth rates. There, we identified evolutionary optimal reproduction modes—those which maximize the population growth rate. We show that, if a mass is conserved upon the reproduction (the mass of parent organisms is equal to the combined mass of offspring), the optimal reproduction mode is fragmentation into exactly two parts. Numerical simulations demonstrated that these two parts should be typically equal in mass. However, fragmentation into unequal parts is optimal when an organism grows fast only in a narrow range of masses. We additionally show that fragmentation into more than two parts can be evolutionarily optimal if some biomass is lost during reproduction. Finally, we identify scenarios where the optimal maturity mass evolves to an arbitrarily large value, which can constitute a path toward simple macroscopic multicellularity.

Model and Methods

We consider a large population of organisms, where the "organism" may represent a single cell or a multicellular colony. Each organism is characterized by a single parameter–its mass m. An organism grows by acquiring resources from the environment. The rate of growth depends on the mass, and, in the general case, can be represented as

$$\frac{dm}{dt} = g(m). \qquad [1]$$

The organism growth function $g(m)$ is determined by the interactions between the organism and its environment, and we consider it to be independent of interactions with other organisms in the populations (density and frequency independence).

In a population, new organisms emerge due to the fission of existing organisms. Deterministic life cycles (34), in which reproduction follows the same pattern for every organism in a population, are common in nature. Therefore, we assume that the fragmentation always occurs upon reaching the critical mass m^*, when an organism instantly fragments into a set of z smaller offspring with masses $\mathcal{M} = \{m_1, m_2, \ldots, m_z\}$; see Fig. 1. Since mass cannot be gained during reproduction, any life cycle $\mathcal{M}$ is restricted by

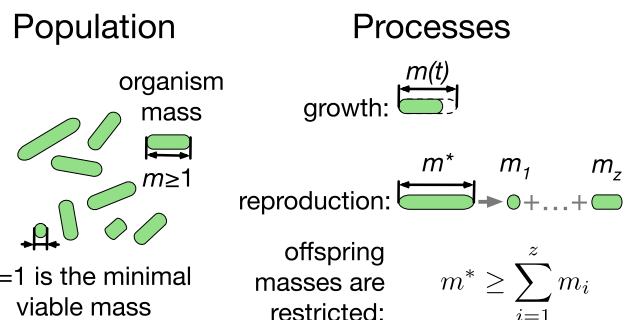$$\sum_{i=1}^{z} m_i \leq m^*. \qquad [2]$$

Fig. 1. The population is composed of organisms, which grow and reproduce. The model considers a population of organism, each characterized by its mass m. Each organism can be changed by two processes: growth and reproduction. In the course of growth, the rate of mass accumulation ($g(m)$) is mass dependent; see Eq. **1**. Reproduction occurs when the organism reaches the critical mass m^*, where it fragments into a number of smaller organisms with masses $m_1, \ldots, m_z$, such that the combined mass of offspring does not exceed the parental mass, $\sum_i m_i \leq m^*$. The mass of offspring has a lower bound $m_i \geq 1$, as the minimal viable mass of an organism is set to one.

We additionally assume that, for each species, there is a minimal possible mass of an organism, below which life cannot be sustained. We use this minimal viable mass as a unit of measurement, so that

$$m_i \geq 1. \qquad [3]$$

As a result of organism growth and fragmentation, the population continuously grows in numbers. In *SI Appendix*, section S1, we show that, under the rules described above, the stationary regime of the population dynamics is an exponential growth with the growth rate λ provided by the solution of a special case of Euler–Lotka equation (14, 45–48) in the form

$$\sum_{i=1}^{z} e^{-\lambda T(m_i, m^*)} = 1, \qquad [4]$$

where

$$T(m_i, m^*) = \int_{m_i}^{m^*} \frac{1}{g(y)} dy \qquad [5]$$

is the time it takes for an organism to grow from one of the initial masses m_i to the maturity mass m^*. For a given organism growth function $g(m)$, the population growth rate λ is a function of the executed life cycle: the maturity mass m^*, the number of offspring z, and the distribution of their masses $\mathcal{M}$.

In the scope of our model, the evolution of life cycles is entirely determined by growth competition: Life cycles with higher population growth rate (λ) outgrow competitors with lower growth rates. As a consequence, the life cycle(s) maximizing the population growth rate λ are evolutionarily optimal–they outcompete any other life cycle.

In this work, we investigate what kind of life cycles are evolutionarily optimal for a range of organism growth functions $g(m)$. In our investigation, we optimize the population growth rate (λ) with respect to the maturity mass (m^*), the number of offspring (z), and their mass distribution ($\mathcal{M}$). We restrict the offspring masses to be above the minimal viable mass, Eq. **3**. Additionally, the combined offspring mass is constrained by mass conservation Eq. **2** and hence is not an independent parameter. We can fully characterize a life cycle by the mass distribution of offspring ($\mathcal{M}$). Then, the number of offspring (z) is given by the length of this sequence, and the maturity mass (m^*) is given by the sum of offspring mass (plus the amount of the lost biomass in the case of costly fragmentation).

Below, we show that the combination of a fundamental law of physics, mass conservation according to Eq. **2**, with the central equation of demography Eq. **4** allows us to understand how a diverse set of life cycles of microorganisms can be promoted by natural selection.

Results

Our main theoretical finding is that, when the mass is conserved in the course of reproduction ($\sum_{i=1}^{z} m_i = m^*$), for any growth function $g(m)$, the maximal population growth rate is achieved by some binary fragmentation $\mathcal{M} = \{m_1, m_2\}$ (*SI Appendix*, section S2). If there is a single global optimum of population growth rate, it must be a division of an organism into exactly two parts. In principle, a fragmentation into multiple parts may also achieve the maximal possible growth rate. But, in this case, the optimum is not unique—at least two other life cycles with a smaller number of offspring must have the same growth rate, as well. We identified one such scenario: Under constant productivity per mass unit, $g(m)/m = 1$, all fragmentation modes have the same growth rate $\lambda = 1$; see *SI Appendix*, section S3. There, the efficiency of organism growth is independent of the organism size, and, thus, selection is indifferent to the reproduction mode. In other words, the constant productivity constitutes evolutionary neutral conditions. In all other cases considered in this study, we found a single global optimum.

Below, we consider the organism growth patterns in terms of productivities ($g(m)/m$) rather than growth functions ($g(m)$), so that advantageous (high productivity) organism sizes are easily identifiable.

Unicellular Organisms Split into Two Equally Sized Offspring. Solitary living cells come in many shapes (49, 50), most prominently, spherical and rod-shaped cells. The shape of a cell has an impact on cell growth, since nutrients are absorbed through the surface, while the processing of these nutrients occurs in a body volume (51). The difference in surface-to-volume ratio between various cell shapes inevitably has an impact on the growth and reproduction patterns of the unicellular species.

From first principles, we model the nutrients' flow into the cell as being proportional to their concentration in the environment (U) and the surface area of the cell. We assume that the processing of absorbed nutrients (substrate) into the biomass occurs via Michaelis–Menten kinetics, where free enzymes reversibly form complexes with substrate, and these complexes are transformed into the biomass of the cell. This biomass, in turn, is divided between enzymes $E_0(m)$ and a constant inert component M_0,

representing nonprocessing parts such as DNA; see Fig. 2*A* and *SI Appendix*, section S4 for details.

We found that, among all possible modes of a rod-shaped or spherical cell reproduction, the best mode is an equal split; that is, the cell grows to size m^* and subsequently splits into two units of size $m^*/2$. To find this, we numerically investigated evolutionarily optimal reproduction modes of spherical and rod-shaped cells in differently nutritious environments. In all cases, evolutionary optimal life cycles have the form $\mathcal{M} = \{m^*/2, m^*/2\}$. The amount of available nutrients influences the optimal mass at which cell division occurs (m^*): More nutrients lead to larger cell mass. This is in line with the widely known observation that bacterial cells in the exponential phase of growth, when food is abundant, are larger than in the stationary phase, when food is depleted (52, 53).

The Smallest and the Largest Cells Are Rod Shaped. The optimal cell masses at the division differ between spherical and rod-shaped cells; see Fig. 2*B*. The most striking prediction of our model is that, for rod-shaped cells in a nutrients-rich environment, the optimal strategy is to grow infinitely without breaking of a cell into parts. The population growth rate (λ), however, remains limited; see Fig. 2*C*. At first glance, a "life cycle without reproduction" may look very counterintuitive. Still, a growth of the cell into a single long filament of a macroscopic scale (often with multiple nuclei) can be interpreted as an example of such a reproductive strategy. This is a common life history strategy among a number of species: Molds growing on a decaying food source (54) or filamentous bacteria occupying the gut of rodents (55) are good examples.

In the opposite case of a nutrient-poor environment, cell shapes tend to be smaller. Our model predicts that, under scarcity of resources, rod-shaped cells develop the smallest cell mass; see Fig. 2*B*. This is in line with the observation that the smallest free-living bacterium, *Pelagibacter ubique*—a major component of the phytoplankton—has the shape of a (slightly bent) rod (56). Additionally, our model puts the lower bound of biomass for a rod-shaped cell as $\min(m^*)_{\text{rod}} = 1.5 M_0$ and, for a spherical cell, as $\min(m^*)_{\text{sph}} \approx 2.92 M_0$; see *SI Appendix*, section S5.

Rosette Colonies Divide by Equal Split. We use the term "rosette colonies" to describe the morphology observed among some simple multicellular species. Rosette colonies are spherical monolayer
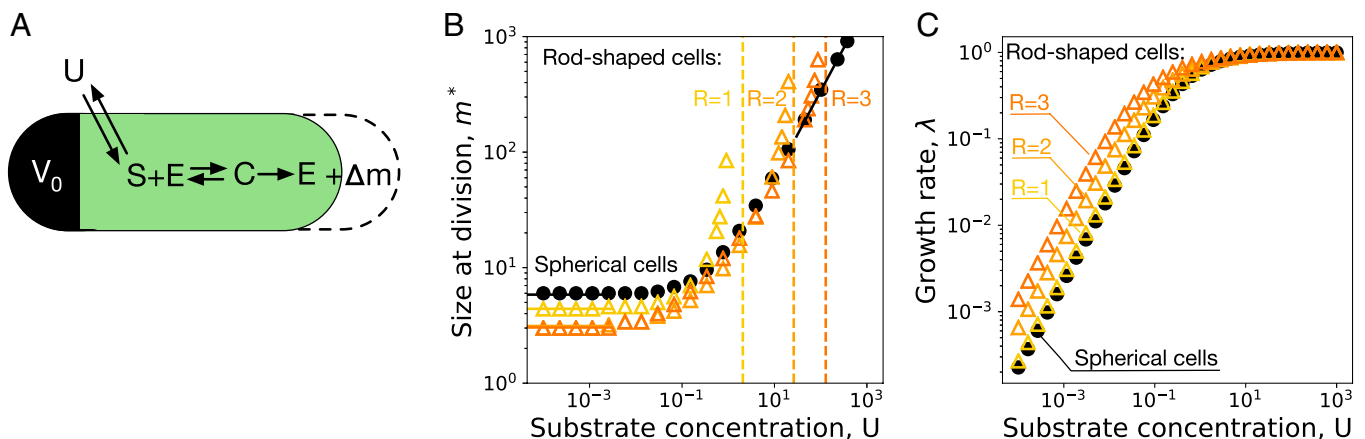

Fig. 2. The largest and the smallest cells are rod shaped. (*A*) Michaelis–Menten kinetics of the cell growth. Nutrients from the environment (*U*) diffuse into the cell. Nutrients inside the cell (*S*) act as a substrate to free enzymes (*E*). They reversibly bind together, forming complexes (*C*), which turn into the biomass Δm, releasing free enzymes. The biomass of the cell is composed of nonprocessing components $M_0 = const$ and enzymes $E_0(m) = E + C$. (*B*) The optimal cell division mass of both spherical (black circles) and rod-shaped (colored triangles) cells grows with the increase of nutrient concentration in the environment *U*. For rod-shaped cells, this mass has a vertical asymptote (dashed lines), beyond which the optimal life history strategy is to propagate the filament without breaking (*SI Appendix*, section S5). For spherical cells, the optimal mass at division follows a power law and, hence, always remains finite. For low nutrient concentration, rod-shaped cells have a smaller biomass than spherical cells. (*C*) The population growth rate λ remains finite in any environment for any cell shape. Rod-shaped cells always have a higher population growth rate than spherical cells, as they have a higher surface-to-volume ratio.

cell clusters, where each cell is attached to the center of the sphere. A number of simple organisms develop rosette colonies in the course of their life cycles, for example, the choanoflagellates *Salpingoeca rosetta* (43), ocean-living magnetotactic bacteria (5), and the golden algae *Synurales* (57). Besides the colony shape, there is little similarity between these species. Nevertheless, all three share a notable reproduction mode: Rosette colonies split into a pair of smaller daughter colonies of similar size; see refs. 5, 43, and 57 and *SI Appendix*, section S6. Such a reproduction mode requires the coordinated action of multiple cells for reshaping of the spherical colony into two smaller spheres.

However, one can imagine a different mode, with a radial division of a cell, such that a cell farther from the center leaves the colony and grows into another rosette, while the cell closer to the center remains attached to the parental colony. Such an imaginary reproduction mode does not need any collective-level actions. A similar mechanism is used by sedentary forms of the same choanoflagellate *S. rosetta* (43, 58), where one cell becomes a free swimmer while another remains attached to substrate. Thus, the imaginary mode of the colony reproduction can fully rely on preexisting mechanisms of cell reproduction, unlike the equal split. Combining this with other advantages provided by reproduction via single-cell bottleneck such as policing of selfish mutants (reviewed in ref. 59), the production of unicellular offspring should be the primary way of rosette colonies reproduction. Why did a more sophisticated reproduction via colony fission repeatedly evolve in unrelated species, which bear no other similarities except the colony shape?

To use our theory, a profile of productivity $g(m)/m$ has to be defined. However, little is known about the growth of rosette colonies. Mechanisms providing an advantage to rosette colonies over unicellular existence were suggested, but the details of their actual contribution remain unclear (19, 20, 23). Still, the very structure of the rosette provides us a glimpse of what the productivity profile could be. In a rosette, each cell is anchored to the center of the colony. Due to the limited space around the center, the maximal cell count of a rosette colony must be limited [actual rosettes contain from 4 to 50 cells (5, 8)]. Rosettes with a cell count exceeding this limit must experience high mechanical stress applied to member cells, which will harm their functional capacity. Following this idea, we assume that the productivity profile satisfies two conditions:

1) An increase in the colony size increases the productivity while the colony size is below the critical value (representing a completely packed rosette), $d/dm\,[g(m)/m] > 0$ for $m < m_{crit}$.

2) A completed rosette ($m = m_{crit}$) is unable to incorporate any more cells, so further growth leads to a strong decrease in productivity, $d/dm\,[g(m)/m] < 0$ for $m > m_{crit}$, until it reaches zero at $m_{crit} + 1$, $g(m_{crit} + 1) = 0$.

To explore all possible productivity profiles that fulfill these conditions, we generated a large set of random profiles $g(m)/m$ satisfying the conditions above; see *SI Appendix*, section S6 for details. For each profile, we numerically identified the evolutionarily optimal life cycle and analyzed the resulting life cycle set; see Fig. 3. We characterized each optimal life cycle $S = \{m_1, m_2\}$ by a symmetry measure defined as

$$A = 2\frac{m_1 - 1}{m_1 + m_2 - 2}, \qquad [6]$$

where the order of fragments satisfies $m_1 \leq m_2$. For equal split life cycles, $\mathcal{M} = \{m_1, m_1\}$, the symmetry is maximal $A = 1$. For extremely asymmetric fragmentation $\mathcal{M} = \{1, m_2\}$ or $1 < m_1 \ll m_2$, the symmetry takes the lowest value, $A = 0$.

We found that the hard limit on the maximal rosette size strongly promotes equal split fragmentation. Fifteen percent of evolutionary optimal fragmentation modes featured complete symmetry $A = 1$, and 95% had symmetry greater than 0.6; see Fig. 3B. Highly asymmetric fragmentation modes ($A \leq 0.2$) were observed only in 0.036% of the investigated profiles (36 cases out of 10^5). No completely asymmetric life cycles $S = \{1, m_2\}$ were found, and only four cases were observed in which the minimal offspring was smaller than two mass units.

To study what caused such a disparity between two classes of life cycles, we analyzed the productivity profiles promoting
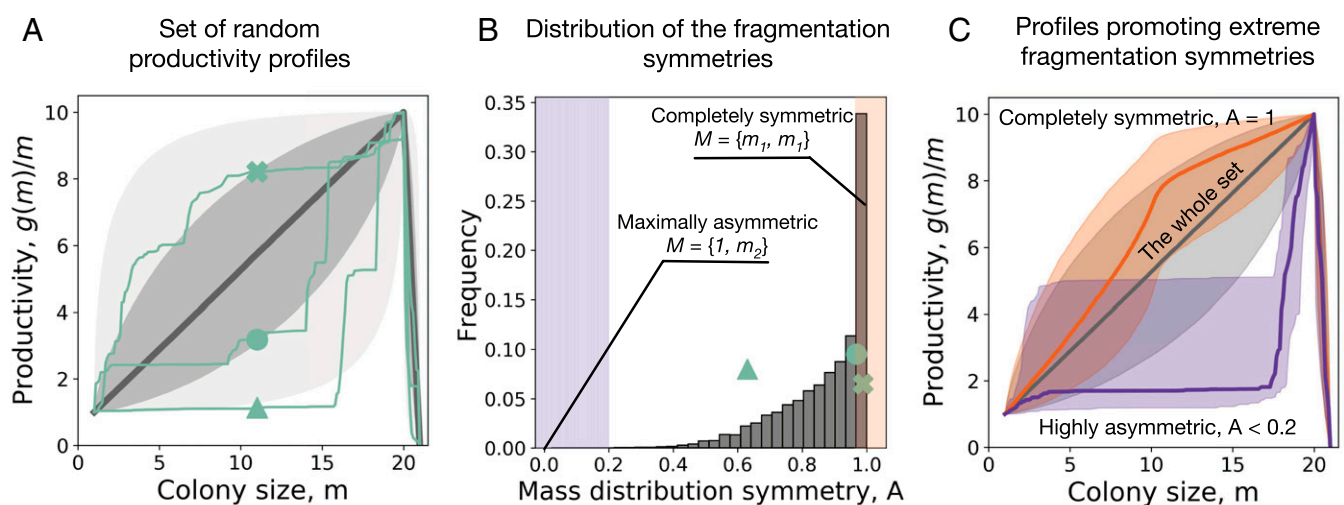

Fig. 3. Random rosettes productivity profiles promote equal split. (*A*) Three productivity profiles (green lines) from the set of 10^5 random profiles. The thick gray line represents the median productivity profile for the set, while gray areas show 50% and 95% CIs for productivity. The critical rosette size was set to $m_{crit} = 20$, where the productivity reached its maximum $g(m_{crit})/m_{crit} = 10$. (*B*) The histogram of symmetry values A among found evolutionary optimal life cycles in this set. The vast majority of found life cycles feature an equal split fragmentation. Green symbols represent the symmetries of the life cycles optimal in the examples presented in *A*. Shaded areas represent the subsamples illustrated in *C*. (*C*) Statistics over profiles promoting highly asymmetric fragmentation ($A < 0.2$, purple) and equal split ($A = 1$, orange), compared against the whole dataset (gray). Lines indicate median profiles, and shaded areas represent 50% CIs. Profiles promoting asymmetrical fragmentation feature a narrow peak of productivity optimality.
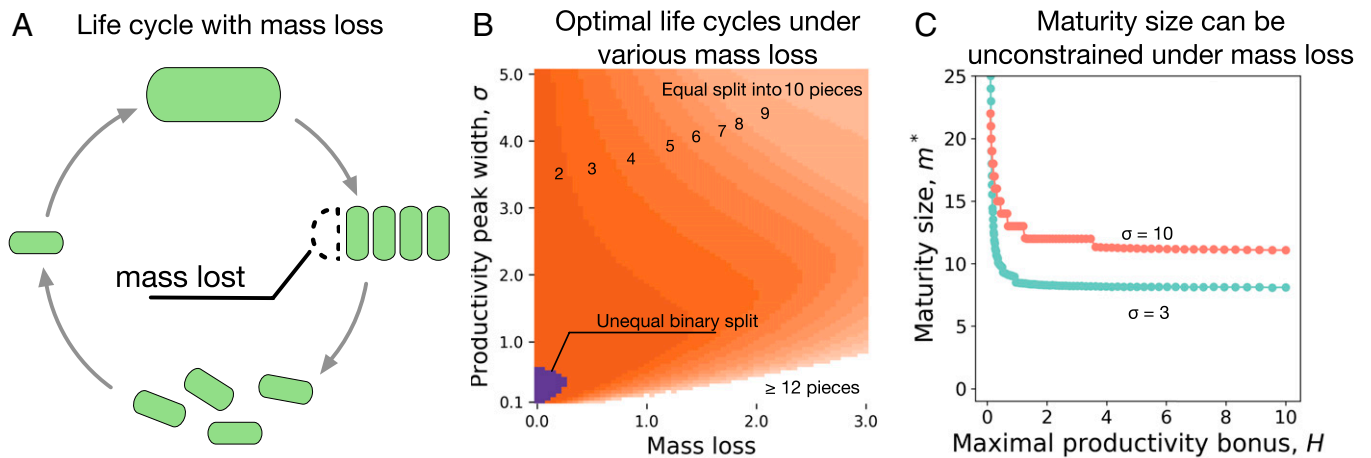
Fig. 4. Microbial life cycles with mass loss. (*A*) Schematic of a life cycle with mass loss at reproduction, such that the combined offspring masses are less than the maternal cell mass. (*B*) When mass gets lost during reproduction, any mode of reproduction can be evolutionarily optimal. However, when productivity has a single optimum, for example, with productivity function $g(m)/m = 1 + He^{-(m-m_{opt})^2/(2\sigma^2)}$, only equal split into multiple fragments and unequal binary splits are found to be optimal. (*C*) If the benefits of size (*H*) are small, the units grow large and leave a lot of offspring, as the optimal life cycle aims to minimize the impact of the reproduction costs. For *B*, we use $m_{opt} = 5.0$, $H = 1.0$. For *C*, we use $m_{opt} = 5.0$, loss = 1.0 of minimal viable mass.

asymmetric fragmentation. We found that, for a life cycle with low symmetry to be evolutionarily optimal, it is necessary to have a plateau of productivity on intermediary colony sizes and a narrow productivity around m_{crit}; see Fig. 3*C*. At the same time, in the case of equal split, productivity profiles did not express notable features, except for a slight tendency toward a concave shape; see Fig. 3*C*. Hence, for asymmetric reproduction to become evolutionarily optimal, it is necessary to satisfy an additional requirement on top of the conditions we posed above; thus the statistical weight of the asymmetric reproduction life cycles is low.

A Loss of Mass Promotes Fission into Multiple Parts. Another widespread pattern of microscopic life cycles is a reproduction with a mass loss (40). Examples are distributed across the whole tree of life: Discarding a maternal cell wall is known among cyanobacteria (60) and green algae (61), and in schizonts of *Apicomplexa* (62) and *Ichthyosporea* (63). Additionally, a sporulation of slime molds—a phenotype that emerged independently in clades *Amoebazoa* (64, 65), *Rhizaria* (66), and *Excavata* (67)—also incurs a biomass loss, because spores leave behind the remnants of the slime mold plasmodium. In all these cases, the act of reproduction gives rise to multiple offspring or spores.

From our theory, it follows that fragmentation into multiple pieces can evolve specifically when some mass is lost upon reproduction ($\sum_{i=1}^{z} m_i < m^*$); see Fig. 4*B*. If the mass loss is large enough, multiple fragmentation gains an advantage over a binary fragmentation, as it allows minimizing of the biomass loss per produced individual. The growth rate profile $g(m)$ determines how large the biomass loss must be in order to make multiple fragmentation optimal. This critical biomass loss can be estimated to be of the order of magnitude of the mass of a minimal viable cell $m = 1$; see *SI Appendix*, section S9. The more organism mass is lost, the more offspring units are produced in the optimal life cycle. Furthermore, when the productivity of the organism is independent on its size $g(m)/m = 1$, our theory suggests that the optimal life cycle consists of growth to the maximally possible size and then fragmentation into minimally viable offspring (Fig. 4*C*; see *SI Appendix*, section S8 for a formal proof). These results align well with the development of macroscopic plasmodium of true slime molds (*Myxomycetes*) (64). Their plasmodium remains flat in the course of its growth and consumes nutrients over its entire surface. As a result of such growth, *Myxomycetes* plasmodium can reach decimeter-size scale, while remaining, formally, a single cell.

This makes a striking difference compared with much smaller (millimeter-scale) slugs of *Dictyostelium discoideum*, which are assembled via aggregation and do not consume nutrients upon formation (65).

Discussion

Nature exhibits a great variety of species with diverse morphological, genetic, and ecological backgrounds. This fascinating diversity makes finding general principles challenging. We developed a theoretical model, which incorporates three factors that are shared among all life forms: 1) the necessity to reproduce, 2) the need to comply with mass conservation law during this process, and 3) evolutionary competition with others. These factors are of special importance for organisms which did not develop high functional and spatial specialization among components—with bacterial cells, unicellular eukaryotes, and undifferentiated multicellularity being the prime examples. Our results show how diverse life cycles became evolutionarily optimal for a wide range of species across the entire tree of life. Maybe the most intriguing of these is that our model suggests two mechanisms for how relatively simple organisms can achieve macroscopic sizes: via unconstrained growth of filaments in rich media (scenario of fungal molds) or via size-independent productivity combined with reproduction costs (possible for *Myxomycetes* slime molds).

Our results state that, when biomass is strictly conserved in the act of fragmentation, the optimal reproduction mode is some form of division in two parts. These parts are not guaranteed to be of the same size, but, in many cases, we found an equal split to be the optimal reproductive strategy. In our study, we considered productivity profiles with a single optimum of organism growth. Equal splits are found to be associated with wide peaks, while unequal splits emerge under narrow productivity peaks; see Figs. 3*C* and 4*B*. When an equal split life cycle is optimal, the organism is born smaller than the optimal size, then it grows and fragments into two equal parts at a size larger than the optimum. Fragmentation at smaller sizes would make the newborn states less efficient, while fragmentation at larger sizes would make the mature organism grow slower. The balance between these two factors determines the optimal equal split. However, if the optimality peak is narrow, then both the newborn and the mature organisms may grow slowly. In such a case, an unequal split becomes optimal, where the larger offspring unit is only slightly smaller than the optimal

size. This offspring quickly grows to a size slightly larger than the optimum, at which moment it divides asymmetrically, returning back and producing a much smaller offspring. In this case, the population growth is driven by rapidly reproducing large organisms. Beyond the examples considered in our work, we speculate that multimodal productivity profiles (with multiple peaks) may also promote unequal split because different offspring may utilize productivity peaks at different organism sizes. Unequal splits are observed in nature, with a prime example being the model organism *Saccharomyces cerevisiae* (budding yeast) reproducing by budding. Under which conditions an unequal split fragmentation becomes an evolutionarily optimal reproductive strategy remains an interesting open question.

The question of what reproductive strategy is evolutionarily optimal has been extensively studied in classic life history theory (10–15). Yet, this theory traditionally focuses on complex multicellular species, and a number of assumptions satisfied by macroscopic beings cannot be transferred to microbial populations. For example, the offspring produced in the act of reproduction of a multicellular entity is easily distinguished from the parent organism and does not inherit any of the functional biomass of the parent. By contrast, among microbes, the parent organism typically ceases to exist in the result of reproduction (cell division), and its biomass is distributed among the offspring. The life histories of multicellular species are largely dictated by their internal developmental program: How long they take to mature, how large the offspring is, and how many offspring are produced are internal features of a given species. The developmental plans of microscopic beings are much simpler, but they feature a greater plasticity with respect to environmental conditions: Microbial cell sizes vary with abundance of nutrients, and even the reproduction itself might be triggered by changes in the environment. As a consequence of their simplicity, microscopic species are potentially capable of executing a large diversity of reproductive strategies: binary and multiple fragmentation into equal and nonequal pieces. In this work, we study how the vast space of possible reproduction modes is constrained by the most fundamental trade-off: mass conservation. The theory presented here demonstrates that mass conservation (Eq. **2**) not only naturally complements the fundamentals of classic life history theory (Eq. **4**) but also leads to easily interpretable results, provides explanations of why a number of naturally observed life cycles are evolutionarily advantageous, and is able to provide a testable hypotheses about the evolution of microscopic life cycles.

The present model makes use of a number of assumptions as we consider a well-mixed population allowed to grow without constraints. While, in some cases, the environmental structure plays a role in the life cycle [e.g., in life cycles of microbes associated with hosts (68, 69)], there are still a huge number of microscopic species with populations that can be well approximated as well mixed: planktonic life forms, soil inhabitants, and many bacterial populations. However, we find examples supporting our results even among species for which dispersal between distant patches plays a significant role: filamentous bacteria in the gut of rodents and molds. Our model predicts that, in resource-rich environments, rod-shaped microorganisms should abstain from reproduction. This is unlike the spherical-shaped organisms, for example, parasitic Ichthyosporea (70), which keep reproducing even in resource-rich environments. In these cases, the efficient exploitation of the current patch of resources (which is optimized in our theory) is an essential prerequisite of the successful dispersal of spores, since the species producing spores earlier and in larger quantities inevitably gain evolutionary advantage at the higher level of population structure.

In our analysis, we do not consider any constraints on the offspring and maturity sizes, other than that they all should exceed minimal viable mass ($m_i > 1$). Such restrictions may, in principle, be in place for various reasons, and they may affect the evolutionary optimality of life cycles. In some cases, a mass restriction might be reflected by our model. For instance, if the maximal possible maturity mass is restricted, this can be implemented by the growth function set to zero above a critical value ($g(m)|_{m>m_{crit}} = 0$), such that no organism can grow above the limit m_{crit}. Similarly, if the minimal possible offspring mass is restricted, this is equivalent to growth function equal to zero below a critical value ($g(m)|_{m<m_{crit}} = 0$), such that offspring born below the limit m_{crit} do not contribute to the population growth. In other cases, for example, if the maturity size is limited from below, or offspring size is limited from above, finding the optimal life cycle should take into account these restrictions explicitly and consider a bounded subset of available reproduction modes.

Formally, our basic model considers a population undergoing unconstrained exponential growth. However, this is not a fundamental restriction—a more sophisticated model with a population size constraint provides exactly the same evolutionarily optimal reproduction modes; see *SI Appendix*, section S7.

We also assume that the organism growth rate depends only on its size—any two organisms of the same mass grow identically. This assumption does not hold if the organism reproduction yields qualitatively different offspring. The simplest example here is the sedentary bacterium *Caulobacter crescentus*, which is typically attached to a solid surface by a stalk. Upon reproduction, only one offspring keeps the stalk, while another develops a flagellum and becomes a motile cell, which will later attach to the surface at another location (71). The two daughter cells differ in their interactions with the environment (sessile and motile) and hence grow at different rates. Another example is the asymmetry with respect to biomass and cell membrane age observed in a wide range of species: budding yeast (72), *C. crescentus* (73), *Bacillus subtilis* (74), and *E. coli* (75). In all these cases, the cells inheriting older components experience a decrease in their growth rates. This leads to the aging of cells in the population. In budding yeast, the cell lineage inheriting the older components ceases to divide in about 24 generations (72). In *E. coli*, the cells inheriting the older pole of the mother cell experience a decrease in division rate (relative to the whole population) of about 1% per generation (75). While our current model does not take into account this effect of inherent asymmetry, its impact on the evolution of microbial life cycles is a very interesting open question.

Another assumption we made is that the organism is capable of executing the chosen reproduction mode with perfect accuracy. In the stochastic world of microorganisms, perfect accuracy cannot be achieved. For instance, measurements of the equality of the binary cell division in rod-shaped bacteria demonstrated that the position of the Z ring, which initiates the formation of the division septum, may deviate from the middle of the cell by 2 to 3% of the cell length (76, 77). The mass of the cell at the moment of division also varies (78). However, microbial species also possess mechanisms compensating for these fluctuations: Too large cells tend to grow less before cell division, while too small cells are allowed to grow more before dividing again, to catch up with the rest of the population. This is facilitated by an elegant "adder" character of the cell cycle control: Cells add the same amount of volume between divisions (79). This way, the offspring sizes are distributed more narrowly than the parent sizes, and the whole population keeps the sizes in homeostasis. From this perspective, our model ignores the individual

variations in life cycle and shows the evolutionary optimal homeostatic strategy.

While our model is designed with microscopic organisms in mind, there are no restrictions on the scale of focal organisms. We expect that our approach is also relevant to larger objects, which are capable of partitioning their mass, for example, to simple social colonies without division of labor.

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