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The evolutionary path to terminal differentiation and division of labor in cyanobacteria

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ABSTRACT

A common trait often associated with multicellularity is cellular differentiation, which is a spatial separation of tasks through the division of labor. In principle, the division of labor does not necessarily have to be constrained to a multicellular setting. In this study, we focus on the possible evolutionary paths leading to terminal differentiation in cyanobacteria. We develop mathematical models for two developmental strategies. First, of populations of terminally differentiated single cells surviving by the exchange of common goods. Second, of populations exhibiting terminal differentiation in a multicellular setting. After testing the two strategies against the effect of disruptive mutations (i.e. "cheater" mutants), we assess the effects of selection on the optimization of the ratio of vegetative (carbon fixing) to heterocystous (nitrogen fixing) cells, which in turn leads to the maximization of the carrying capacity for the population density. In addition, we compare the performance of differentiated populations to undifferentiated ones that temporally separate tasks in accordance to a day/night cycle. We then compare some predictions of our model with phylogenetic relationships derived from analyzing 16S rRNA sequences of different cyanobacterial strains. In line with studies indicating that group or spatial structure are ways to evolve cooperation and protect against the spread of cheaters, our work shows that compartmentalization afforded by multicellularity is required to maintain the vegetative/heterocyst division in cyanobacteria. We find that multicellularity allows for selection to optimize the carrying capacity. These results and the phylogenetic analysis indicates that terminally differentiated cyanobacteria evolved after undifferentiated species. In addition, we show that, in regimes of short daylight periods, terminally differentiated species perform worse than undifferentiated species that follow the day/night cycle; indicating that undifferentiated species have an evolutionary advantage in regimes of short daylight periods.

1. Introduction

1.1. Multicellularity and the germline–soma divide

Multicellular organisms undergo cellular differentiation in order to perform distinct tasks. A fundamental example is differentiation into germline and somatic cells. This division of labor was first elucidated by Weismann (1889) upon studying aquatic animals such as hydrozoans, and green algae of the order Volvocales (Schleip, 1934). He distinguished between germ cells (*Keimzellen*) that contribute cells and hereditary material to the subsequent generation of a multicellular individual, and somatic cells (*Somatische Zellen*) that help in the survival of an individual during its lifetime. In some animals, differentiation into germ cells can be irreversible, referred to as "terminal differentiation." The germline–soma divide is now viewed as a fundamental organizational scheme in complex multicellular organisms, and is central to understanding the interplay between natural selection at the level of the multicellular individual, and competition between its component cells (Buss, 1983, 1988).

The separation between a germline and soma is not unique to Eukaryotes, and is also mirrored in differentiated multicellular cyanobacteria (Saier and Jacobson, 1984). The latter can differentiate into vegetative and heterocystous cells, which are functionally equivalent to germline and soma, respectively. Moreover, differentiation into heterocystous cells is terminal. The fact that the same fundamental organizational scheme for the division of labor has independently appeared in such disparate lineages suggests that there may be general conditions that favor the emergence of such an organization. With this view in mind, multicellular cyanobacteria can serve as a model organism for understanding the developmental and ecological conditions that lead to the evolution of terminal differentiation and a germline–soma divide.

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Although there is a growing literature on modelling the ecology and population dynamics of nitrogen fixing cyanobacteria (Tilman, 1977; Roussel et al., 2000; Rabouille et al., 2006; Agawin et al., 2007), the factors that can affect the evolution of multicellularity and differentiation in these organisms has not been examined. In this work we try to approach several fundamental questions. First, we ask what are the fundamental conditions necessary for the evolutionary stability of a terminally differentiated soma in cyanobacteria. Second, we ask how differentiation is related to fitness, and how the rate of differentiation can be optimized in an evolutionary context. Third, we address some of the ecological conditions that may favor the spatial vs. temporal separation of tasks between cyanobacterial cells. Fourth, we examine the phylogenetic history of cyanobacteria in light of our theoretical results.

In the rest of this introduction we discuss the empirical and theoretical background necessary for the models that we subsequently develop.

1.2. Multicellularity in cyanobacteria

The cyanobacteria encompass both unicellular and multicellular species, and are among the most ancient multicellular organisms known (Schopf, 1994). Among multicellular species, differentiation into heterocystous forms seems to have a monophyletic origin (Turner et al., 1999; Seo and Yokota, 2003; Tomitani et al., 2006). Multicellular cyanobacteria such as members of the genera *Anabaena* and *Nostoc* are often present as filaments differentiated into two kinds of cells: vegetatives and heterocysts (Wolk, 1996). Some species also have akinete cells specialized for surviving harsh conditions (hence being similar to spores in their function). We will not deal with akinetes in this study. Vegetative cells are photosynthetic and reproduce by cell division, giving rise to either vegetative or heterocystous cells. They use solar energy and carbon dioxide for the purpose of carbon fixation, and fixed nitrogen in the form of nitrates for building molecules such as amino acids. Fixed nitrogen is produced by heterocysts, whose main task is nitrogen fixation using free atmospheric nitrogen. Heterocysts cannot divide and originate from the division of vegetative cells (a portion of vegetative divisions leads to heterocysts instead of vegetative cells). The need for division of labor between cells that either fix nitrogen or carbon arises from inhibitory chemical interactions between photosynthesis and nitrogen fixation. By having the two chemical reactions occur in different cells, filamentous cyanobacteria can improve the efficiency of nitrogen fixation. In undifferentiated cyanobacteria such as *Synechocystis* sp. or *Oscillatoria* sp., the main strategy is to have a day and night cycle (circadian rythm) (Stal and Krumbein, 1987; Kondo et al., 1993; Bergman et al., 1997; Kageyama et al., 2006; Kurosawa et al., 2006), according to which photosynthesis and nitrogen fixation are temporally separated. The interactions among vegetatives and heterocysts can be also framed in the context of cooperation. Heterocysts sacrifice the possibility of reproduction and fix nitrogen for all the cells, in this sense being a fully altruistic entity. Vegetative cells are also cooperative: they do not use all their progeny to pass their genes to the next generation, because part of it will become heterocystous and will lose this ability. If vegetative cells produce few or no heterocysts in order to maximize their reproductive success, they act as defectors.

A detailed classification of the cyanobacteria has been made by Rippka et al. (1979). Cyanobacteria are phenotypically classified into five sections (I–V), which are schematically depicted in Fig. 1. In the case of heterocystous section IV species such as *Anabaena* sp., it has been recently established that filaments are truly

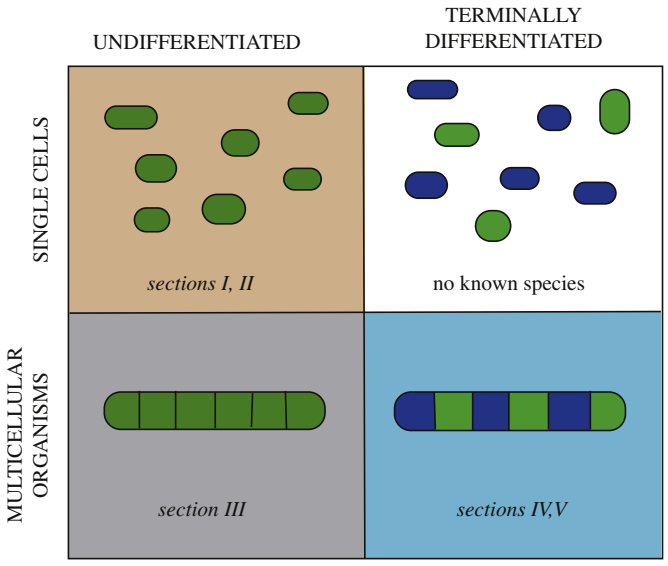

Fig. 1. Schematic classification of cyanobacterial species based on Rippka et al. (1979).

multicellular, in the sense that the periplasmic space along the filament is continuous (Flores et al., 2006; Mariscal et al., 2007). This allows for an exchange conduit for nutrients and other molecules between cells. Given that cyanobacteria are gram negative and possess two membranes, the continuity of the periplasm is achieved via the outer membrane, which forms a unified compartment around a chain of cells, rather than individual cells. Each cell is in turn also encapsulated by its own cytoplasmic membrane. In addition, there is some evidence for direct exchange between the cytoplasms of adjacent cells through membrane channels (Mullineaux et al., 2008).

1.3. Evolution of multicellularity and cooperation

The evolutionary transition between unicellular and multicellular forms involves conflicts between different levels of selection (Buss, 1988; Smith and Szathmáry, 1995; Rainey and Rainey, 2003; Michod, 2007). The benefits associated with multicellularity may for example include size, nutritional advantages, collective protection against antagonists, and division of labor (Shapiro, 1998; Bonner, 2000; Kaiser, 2001). However, multicellular organization does not automatically imply the existence of differentiation. Undifferentiated multicellularity can have its own advantages over single-celled organization (Pfeiffer et al., 2001; Pfeiffer and Bonhoeffer, 2003; Willensdorfer, 2009).

Once multicellularity has evolved, one can consider the conditions under which cellular differentiation would be advantageous. For example, the division between germline and soma can be analyzed as a consequence of the interplay between two fitness components, namely reproduction and survival (Weismann, 1889; Michod et al., 2006). Cooperation among cells is fundamental in building a differentiated multicellular organism. Single entities lose the opportunity of selfish reproduction in order to become part of a community of cells. They produce and share nutrients with the others instead of using everything to their advantage, hence increasing the fitness of the multicellular unit (Michod and Roze, 2001). However, such a behavior can be abandoned by defectors (or cheaters), who exploit the cooperative acts but do not contribute to the common good. Following the work of Hamilton (1964a, 1964b), various studies have been made about cooperation and selfish behavior using game theoretic approaches (Smith and Price, 1973; Hofbauer et al., 1979; Smith,

1982; Hofbauer and Sigmund, 1998; Ohtsuki et al., 2006). Non-cooperative or "cheating" behavior is common in many ecosystems: cheaters can exhibit selective advantages over the competitors (Axelrod and Hamilton, 1981; Sachs et al., 2004; Boomsma and Franks, 2006), but can lead to reciprocal extinction or to stable mutualistic associations (Doebeli and Knowlton, 1998; Roberts and Sherrat, 1998; Ferriere et al., 2002). Over-exploitation of a common good by cheaters is often referred to as the "tragedy of the commons" (Hardin, 1968). It is known that some kind of subpopulation grouping is required for resolving this problem. The classic explanations are kin selection (Hamilton, 1964a, 1964b; Smith, 1964; Frank, 1994; Lehmann and Keller, 2006; West et al., 2006) and reciprocity (Trivers, 1971; Axelrod and Hamilton, 1981; Leimar and Hammerstein, 2001; Hammerstein, 2003; Lehmann and Keller, 2006; Suzuki and Akiyama, 2008). Other mechanisms are for example differential dispersal(Enquist and Leimar, 1993; Hochberg et al., 2008), resource supply (Brockhurst et al., 2008), spatial structuring of the population (Nowak and May, 1992; Nowak et al., 1994; Ferriere and Michod, 1996; Nakamaru et al., 1997; Pfeiffer et al., 2001; Pfeiffer and Bonhoeffer, 2003), allowing for the random emergence of association groups (Michod, 1983; Szathmáry and Demeter, 1987; Killingback et al., 2006), or imposing threshold conditions in the rules of the game (Bach et al., 2006). Various aspects of these theories have been validated in microbes (Buss, 1982; Strassmann et al., 2000; Velicer et al., 2000; Rainey and Rainey, 2003; Griffin et al., 2004; Travisano and Velicer, 2004; West et al., 2006). For example, assortment and phenotypic noise can allow the evolution of self-destructive-cooperation in *Salmonella thyphimurium* (Ackermann et al., 2008), while kin selection limits cheating in the slime mold *Dictyostelium* spp. (Buss, 1982; Gilbert et al., 2007).

Hypercycles, which are autocatalytic networks of enzyme reactions are another system where the issue of cheating and the importance of population subdivision arises (Eigen, 1971; Eigen and Schuster, 1977, 1978). Hypercycles are susceptible to invasion by "parasitic" enzymes that have reduced catalytic activity for the replication of their target enzyme. It has been suggested several times (Eigen, 1971; Eigen and Schuster, 1978; Smith, 1979; Eigen et al., 1980; Michod, 1983) that one way to escape the problem of parasite invasion in the latter case would be the evolution of compartments or "protocells" that allow different hypercycles to compete. The "stochastic corrector model" of Szathmáry and Demeter (1987) implements a version of this concept (Smith and Szathmáry, 1995). In a similar vein, an alternative path to achieve population substructuring is the introduction of spatial heterogeneity (Boerlijst and Hogeweg, 1991; Attolini and Stadler, 2006; Sardanyés and Solé, 2006; Fontanari et al., 2006; Hogeweg and Takeuchi, 2003).

2. Methods

At present, there are no known single-celled species of cyanobacteria that terminally differentiate to form collaborative single species consortia as a means to divide labor between nitrogen and carbon fixers (top-right box in Fig. 1). We model the latter hypothetical scenario (single-celled model) and that of differentiated multicellularity (compartmental model, bottom-right box in Fig. 1).

2.1. Mathematical models

2.1.1. The single-celled model

We consider a single-celled model (Fig. 2a) where vegetatives, heterocysts and cheater vegetatives compete for nitrate, fixed carbon and solar energy. The vegetative cells convert the solar energy into chemical energy (fixed carbon), while the nitrate is produced by heterocysts. Vegetative cells divide into vegetative and heterocyst cells in different proportions. The cheaters, when present in the system, produce and consume resources at the same rate as the non-cheater vegetatives, but they produce less heterocysts—or do not produce them at all. In this model, the resources are shared by all cells living in the environment.

We describe the single-celled model with the following system of ODEs:

$$\frac{dN}{dt} = 2aH\frac{C}{C+k} - p_3N - r(V+V')Z - q\frac{N}{N+k}(V+H+V')$$

$$\frac{dV}{dt} = -p_3V + p_vVZ$$

$$\frac{dH}{dt} = -p_3H + p_hVZ + p_{h'}V'Z$$

$$\frac{dV'}{dt} = -p_3V' + p_{v'}V'Z$$

$$\frac{dC}{dt} = c_eZ_L - p_3C - r(V+V')Z - q\frac{C}{C+k}(V+H+V') - aH\frac{C}{C+k} \qquad (1)$$

where

$$Z = Z(N,C) = \frac{r_0}{\frac{1}{k_0} + \frac{1}{k_CC} + \frac{1}{k_NN} + \frac{1}{k_{NC}NC}}$$

$$Z_L = Z_L(I,G) = \frac{r_0}{\frac{1}{k_0} + \frac{1}{k_II} + \frac{1}{k_GG} + \frac{1}{k_{IG}IG}}$$

I = irradiance (constant)

$G = V + V'$ (photosynthetic units)

$$p_v + p_h = 1, \quad p_{v'} + p_{h'} = 1 \qquad (2)$$

In (1), N is the nitrate concentration (mol/cm^3), V, H and V' are the concentrations of resident vegetative, heterocyst and cheaters cells, respectively (cells/cm^3) and C (mol/cm^3) the concentration of chemical energy (sugar in the form of glucose).

The equations have been built on and can be explained by the following assumptions:

(i) *Reproduction and housekeeping*: The parameter p_v indicates the proportion of vegetative cells originated at any reproduction event. Cheater cells behave similar to vegetative cells, except for their p_v value, designated as $p_{v'}$. Heterocysts are produced in proportion p_h by vegetatives and in proportion $p_{h'}$ by cheaters. Vegetative cells use chemical energy to support dividing ($-rVZ$) and living costs ($-qV(C/C+k)$). Heterocyst cells use chemical energy for nitrogen fixation ($-aH(C/C+k)$) and living costs ($-qH(C/C+k)$). We assume the same death rate p_3 for all cells. The reproduction of vegetatives is regulated by $Z = Z(N,C)$, a Michaelis–Menten type saturation function for two substrates (here nitrogen and sugar).

(ii) *Energetics of nitrogen fixation*: Heterocysts are responsible for nitrogen fixation, which requires about 19 molecules of ATP. Taking into account that a molecule of glucose gives roughly 38 ATP molecules (Lawlor, 1990), we have

$$\frac{38\text{ATP/glucose}}{19\text{ATP/fixed N}} \simeq 2 \cdot \text{fixed N/glucose}.$$

This relation is the basis for an assumed ratio of 2 molecules of nitrogen produced for every glucose consumed. Nitrogen

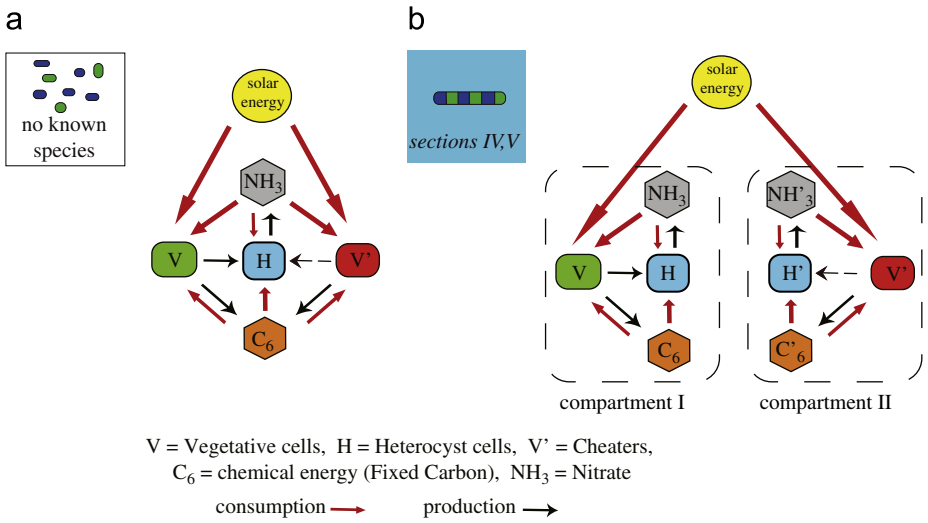

Fig. 2. Schematic representation of interactions between the variables of the models.

fixation is only possible whenever carbon (sugar) is available and it is limited by a saturation function dependent on sugar. It is assumed that free nitrogen is not a limiting factor. Decrease in nitrate is due to natural decay ($-p_3N$), to housekeeping or living cost ($-q(N/N+k)(V+H)$) and to reproduction of vegetative cells ($-rVZ$).

(iii) *Light harvesting*: The solar irradiance, I, is treated as a static parameter, as is common practice in basic models of photosynthesis (Han, 2001; Rubio et al., 2003). The irradiance is absorbed and transformed into chemical energy (six-carbon-sugar) by vegetative and cheater cells. The total production of sugar depends on I and on the total concentration of photosynthetic units G (PSU). We use the function $Z_L = Z_L(I,G)$ to describe the connection between light harvesting and sugar production. In Z_L, the solar irradiance I is absorbed by the photosynthetic units $G=(V+V')$ present in both the normal and cheater vegetative cells, hence I and G are considered as substrates for Z_L. This type of saturation function ensures that whenever a substrate is limiting the considered cellular activity, the potential increase of the other substrate does not enhance the activity. The solar energy is converted into carbon at a rate c_e. We assume that CO_2 concentrations are not a limiting factor. Carbon is subject to a natural decay ($-p_3C$).

(iv) *Carbon to nitrogen consumption ratios*: During exponential growth, the average ratio of carbon to nitrate is roughly C : N $\simeq$ 6 : 1 (Vrede et al., 2002) in a bacterial cell. The uptake of carbon should hence be 6 times higher than that of nitrate. However, considering that a molecule of glucose contains six carbon atoms, we have C : N $\simeq$ 1 : 1. The parameters r and q represent the rate of uptake of N and C for reproduction and housekeeping, respectively. Based on a 1:1 expectation for C:N content, the coefficients for C:N consumption for both reproduction and housekeeping are assumed to be one.

2.1.2. The compartmental model of multicellularity

In the compartmental model (Fig. 2b), only the solar energy is shared, while different compartments produce and consume their own sugar and nitrate units. The cheater is now an aggregate in which the proportion of vegetative cells produced at each division is higher than in the other. The compartmental model is a simplified representation of multicellularity, in which each compartment represents a distinct multicellular individual. Both compartments compete for the same solar energy source, but each has its own cells, nitrate and chemical energy. The following ODE set describes the dynamics of the compartmental model:

$$\frac{dN}{dt} = 2aH\frac{C}{C+k} - p_3N - rVZ - q\frac{N}{N+k}(V+H)$$

$$\frac{dV}{dt} = -p_3V + p_vVZ$$

$$\frac{dH}{dt} = -p_3H + p_hVZ$$

$$\frac{dC}{dt} = \frac{V}{V+V'}c_eZ_L - p_3C - rVZ - aH\frac{C}{C+k} - q\frac{C}{C+k}(V+H)$$

$$\frac{dN'}{dt} = 2aH'\frac{C'}{C'+k} - p_3N' - rV'Z' - q\frac{N'}{N'+k}(V'+H')$$

$$\frac{dV'}{dt} = -p_3V' + p_{v'}V'Z'$$

$$\frac{dH'}{dt} = -p_3H' + p_{h'}V'Z'$$

$$\frac{dC'}{dt} = \frac{V'}{V+V'}c_eZ_L - p_3C' - rV'Z' - aH'\frac{C'}{C'+k} - q\frac{C'}{C'+k}(V'+H') \qquad (3)$$

where

$$Z = Z(N,C) = \frac{r_0}{\frac{1}{k_0} + \frac{1}{k_CC} + \frac{1}{k_NN} + \frac{1}{k_{NC}NC}}$$

$$Z' = Z'(N,C) = \frac{r_0}{\frac{1}{k_0} + \frac{1}{k_CC'} + \frac{1}{k_NN'} + \frac{1}{k_{NC}N'C'}}$$

$$Z_L = Z_L(I,G) = \frac{r_0}{\frac{1}{k_0} + \frac{1}{k_II} + \frac{1}{k_GG} + \frac{1}{k_{IG}IG}} \qquad (4)$$

I = irradiance (constant)

$G = V+V'$ (photosynthetic units)

$p_v + p_h = 1, \quad p_{v'} + p_{h'} = 1$

Variables N, V, H, C, respectively, represent nitrate, vegetatives, heterocysts and chemical energy concentrations of the first

filament, respectively, while N', V', H', C' represent the corresponding variables for the second compartment. The functions Z and Z' have the same meaning as in the single-celled model, except that in this case, they are functions of the respective nitrate and chemical energy of the two compartments. Competition for light between compartments is expressed in the function Z_L, by the variable $G = V + V'$. Light harvesting is due to both compartments, but the terms $V/V+V'$ and $V'/V+V'$ indicate that the income of sugar into the different aggregates is mediated by the concentration of photosynthetic units belonging to the corresponding compartments, hence allowing competition for light. The same assumptions (i)–(v) for system (1) listed in Section 2.1.1 hold also for system (3).

2.2. Numerical analysis of the models

Due to the high nonlinearity of the equations, we do not derive the analytical expression of the steady states of the system nor do we analytically carry out stability analysis. However, numerical simulations indicate that both systems can evolve towards three different kinds of steady state: one corresponding to the extinction of resident and cheater populations (Y_1), one in which only the resident population survives (Y_2), and one in which the cheaters overcome the resident population (Y_3). Parameters listed in Table 1 have been used in the simulations as default parameters for a low irradiance case. Structural stability of the models has been tested by random sampling of other parameter values and initial conditions in $\mathbb{R}^{21}$ and in $\mathbb{R}^{22}$ for the single-celled and compartmental model, respectively (results in Supplementary Information). Numerical integration has been performed using a variable order solver based on linear implicit multistep methods, implemented in function `ode15s` of Matlab (http://www.mathworks.com/).

2.3. Evolutionary stability against cheaters

Using the models in Section 2.1, we simulate competitions between a resident population and either pure or partial cheaters. Mutation is introduced in the systems in the following ways. In the case of pure cheaters, the latter are considered as the mutant. In the single-celled model, mutants are present in the mixed population from the beginning in a given proportion. In the compartmental model, they are introduced in only one of the compartments, while the other one is preserved. In the case of partial cheaters, in both models and for each mutational event, the strain with a p_v different from the resident strain is considered as the mutant.

2.3.1. Evolutionary optimization of vegetative/heterocyst ratio

We consider competitions between strains that differ in their p_v value, with $0 < p_v < 1$. We simulate consecutive competitions between a resident strain (wild type) and a newly arrived mutant. Each step of the simulation is a mutational event, in which after the competition, the winner strain establishes its p_v value as the wild type for the next generation (see Supporting Information for details of the algorithm).

2.4. Division of labor in time and space: periodic vs. differentiated cyanobacteria

We model a population of undifferentiated cyanobacteria subject to day/night irradiance cycle by the following ODE system:

$$\frac{dN}{dt} = 2a\delta_n \frac{C}{C+k}V - p_3 N - rVZ - q\frac{N}{N+k}V$$

$$\frac{dV}{dt} = -p_3 V + VZ$$

$$\frac{dC}{dt} = c_e Z_L - p_3 C - rVZ - (a\delta_n + q)\frac{C}{C+k}V \tag{5}$$

where

$$Z = Z(N,C) = \frac{r_0}{\frac{1}{k_0} + \frac{1}{k_C C} + \frac{1}{k_N N} + \frac{1}{k_{NC} NC}}$$

$$Z_L = Z_L(I,V) = \frac{r_0}{\frac{1}{k_0} + \frac{1}{k_I I} + \frac{1}{k_V V} + \frac{1}{k_{IV} IV}} \tag{6}$$

$$I(t) = A\frac{(\rho(t)+1)^\gamma}{(m^\gamma + (\rho(t)+1)^\gamma)} \tag{7}$$

$$\rho(t) = \sin\left(\frac{\pi t}{12}\right) \tag{8}$$

$$\delta_n = 1 - \frac{(\rho(t)+1)^\gamma}{(m^\gamma + (\rho(t)+1)^\gamma)} \tag{9}$$

In (5), N, C, and V are fixed nitrogen, carbon and cell concentrations, respectively. The irradiance I and the nitrogen

Table 1
Parameters values used in the displayed simulations.

Parameter description	Symbol	Value	Unit
Uptake of N and C for reproduction	r	5	mol cells^{-1}
Uptake of N and C for housekeeping	q	0.8	mol cells^{-1} s^{-1}
Uptake of C for N-fixation	a	1	mol cells^{-1} s^{-1}
Decay rate	p_3	0.001	s^{-1}
Irradiance	I	1000	μE cm^{-2} s^{-1}
Rate of energy conversion	c_e	0.8	mol cm^{-3}
Total stoichiometric concentration	r_0	0.1	
First order rate constant	k_0	10	s^{-1}
Nitrate specificity constant	k_N	10	mol^{-1} cm^3 s^{-1}
Carbon specificity constant	k_C	10	mol^{-1} cm^3 s^{-1}
N–C product specificity constant	k_{NC}	1	(mol^{-1} cm^3)^2 s^{-1}
Irradiance specificity constant	k_I	10	μE^{-1} cm^2
PSU specificity constant	k_G, k_V	10	cells^{-1} cm^3 s^{-1}
Irradiance-PSUproduct specificity constant	k_{IG}, k_{IV}	1	cells^{-1} μE^{-1} cm^5
Transformed Hill sine/cosine functions	γ	30	

Abbreviations: E, Einstein; PSU, photosynthetic units.

fixation function δ_n are Hill transformed sine curves that represent the daylight dependent periodicity (Marler et al., 2006). The default parameter values are as in Table 1. In this model, we assume that cells do not have a true internal circadian rhythm, but follow the external day/night alternation. During daylight, the periodic organisms only perform photosynthesis ($I \simeq 1, \delta_n \simeq 0$) because of O_2 inhibition. At night, when the absence of light impede photosynthesis, nitrogen fixation is allowed ($I \simeq 0, \delta_n \simeq 1$). We compare the performance of undifferentiated periodic species in (5) with the multicellular differentiated species, when the irradiance is described with a Hill transformed sine curve as in (7). To model a population of differentiated cyanobacteria subject to a day/night irradiance cycle, we modify system (1) by removing cheaters (V') and using (7) for irradiance:

$$\frac{dN}{dt} = 2a\frac{C}{C+k}H - p_3N - rVZ - q\frac{N}{N+k}(V+H)$$

$$\frac{dV}{dt} = -p_3V + p_vVZ$$

$$\frac{dH}{dt} = -p_3H + p_hVZ$$

$$\frac{dC}{dt} = c_eZ_L - p_3C - rVZ - aH\frac{C}{C+k} - q\frac{C}{C+k}(V+H) \qquad (10)$$

where Z, Z_L and I are as in (6) and (7). As nitrogen fixation is always performed by heterocysts, the function δ_n is not needed. We compare the performance of the two models with different day and night durations, by changing the value of m in I and δ_n. Large and small values of m correspond to long and short dark periods, respectively. We map the values of m into a percentage of daylight (details in Supplementary Information). As the mapping is based on an approximation of the duration of the day, it is not suited to treat neither complete darkness nor absence of darkness. For these cases, we directly set $I = 0$, 1, respectively.

2.5. Phylogenetic analysis of cyanobacteria

For this study, 16S rRNA gene sequences of 37 cyanobacteria and an outgroup were obtained from GenBank (Table 1 in Supporting Information). The ingroup is represented by nine single celled bacteria from clade I, four single celled bacteria from clade II, 14 multicellular bacteria from clade III, seven multicellular heterocyst forming bacteria from clade IV and four branching bacteria from clade V. Our labeling into clades I–V is based on Rippka et al. (1979). *Agrobacterium tumefaciens* was used for outgroup comparison as suggested in previous studies (Honda et al., 1999; Partensky et al., 1999). Details of the analysis are provided in Supporting Information.

3. Results

3.1. Effect of pure cheaters on evolutionary stability

In the single-celled model, introduction of pure cheaters leads to the extinction of the population (Fig. 3a). Cheaters grow faster and subtract resources from the resident population, which eventually starts decaying after reaching an initial peak. Once the normal vegetative cells are extinct, no entity in the system is able to produce nitrate and the cheaters also die. The collapse of the system in the single-celled model is primarily caused by the fact that the resources are shared between organisms.

In the compartmental model, a pure cheater cell gives rise to an aggregate of cells that cannot sustain itself. The compartmentalization afforded by separate multicellular aggregates (i.e. "multicellular" individuals) allows genetically related cells to protect their resources from a cheater invasion in another aggregate. Hence a cheater can destroy the multicellular aggregate that it arises in, but it cannot destroy the whole population (Fig. 3b). The basic dynamics of the models without cheaters are provided in Supplementary Information.

3.2. Effect of partial cheaters on evolutionary stability

Partial cheating refers to the situation in which a mutant vegetative cell produces heterocysts in a smaller proportion than the resident vegetative cells. We investigated the criterion that leads to the success of one genotype over the other, when two competing populations differ in their p_v value. We tested the outcomes of competitions in both models using Monte Carlo simulations, where pairs of p_v and $p_{v'}$ values were sampled randomly in the interval $[0, 1]$. Vegetative cells belonging to strains with p_v and $p_{v'}$ are indicated by V and V', respectively. Vegetative steady states after competitions are shown in Fig. 4. Fig. 4a shows the results for the single-celled model. In this case we find that at a steady state, $V > V'$ when $p_v > p_{v'}$ and similarly, $V' > V$ when $p_{v'} > p_v$. Hence we conclude that in the single-celled model, the winning factor in the competitions is the value of p_v. The strain with the higher p_v outcompete the other. This result holds when randomly sampling through alternative parameter values and initial conditions (see Supporting Information).

When tested for the compartmental model, the latter winning criterion does not hold. Fig. 4b shows that having a higher p_v does

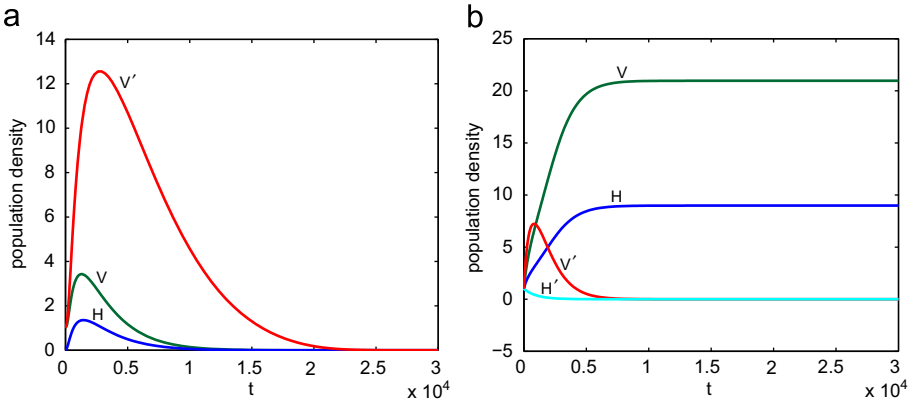

Fig. 3. Effect of pure cheaters on the resident population. V, vegetatives, H, heterocysts, V', cheaters, H', heterocysts in the compartment with cheaters. In (a), all the cell types reach zero, while in (b), cells of the non-mutant compartment can grow and reach a positive steady state.

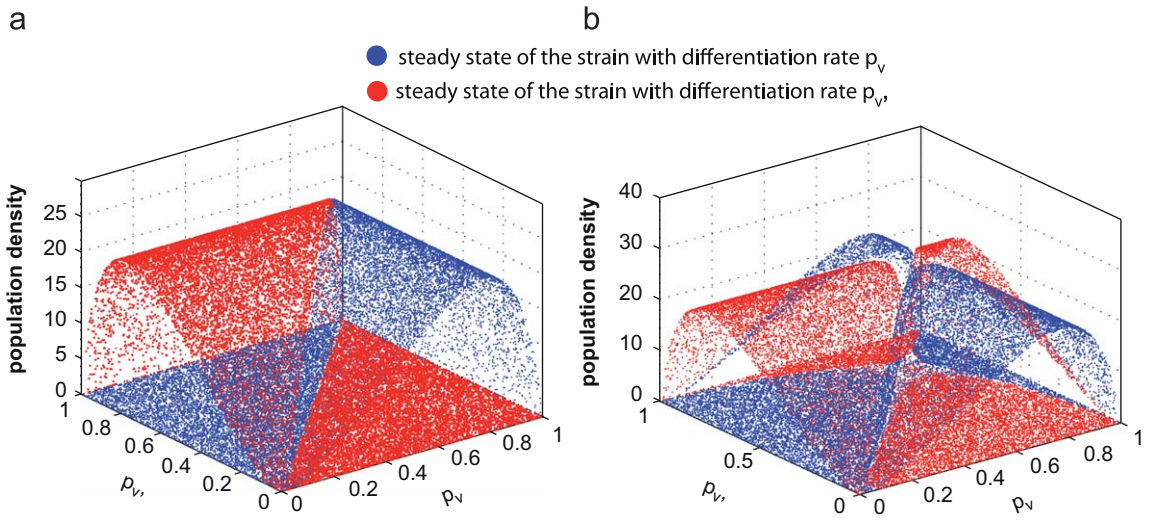

Fig. 4. Steady state density of vegetative cells after competitions between strains differing in their p_v value (plots show representative 15,000 competitions from 115,000). The blue and red dots correspond to strains competing with differentiation rates of p_v (blue) and $p_{v'}$ (red), respectively. (a) In the single-celled model, the strain with the higher p_v wins. Hence, when $p_v > p_{v'}$, the strains with p_v (blue dots) are shown at a higher steady state. When $p_{v'} > p_v$, the red dots are shown at a higher steady state. (b) In the compartmental model, the strain with the higher carrying capacity wins. The ratio of p_v to $p_{v'}$ is no longer the factor that determines which strain wins.

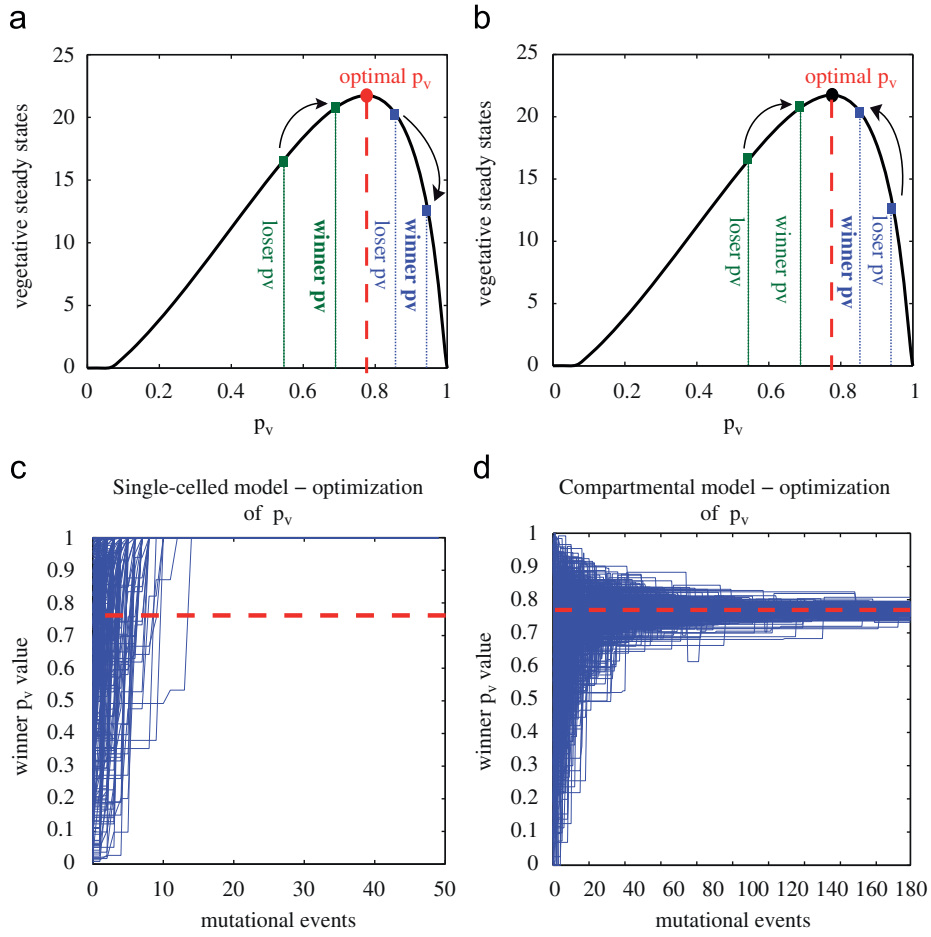

Fig. 5. Optimization of vegetative to heterocyst cell ratios. Plots (a) and (b) illustrate the winning factor in competitions between strains with different p_v. The parabola-shaped curve represents the carrying capacity as function of p_v. For each case, the carrying capacity of two pairs of strains simulated in isolation are shown as examples (green and blue squares). Next to each strain, we indicate the outcome of the competition within the pair (winner or loser). (a) In the single-celled model, the strain with the higher p_v wins. (b) In the compartmental model, the one reaching the higher carrying capacity wins. Plots (c) and (d) show the outcome of repeated mutational events, at which a competition between resident and mutant strain take place. (c) In the single-celled model, the optimal p_v (red line) is surpassed and $p_v \to 1$. (d) In the compartmental model, p_v tends to the optimal value.

not play a role anymore. In the multicellular organization, the winning indicator is the vegetative steady state value that would be reached with the corresponding p_v or $p_{v'}$ value in isolation. This winning indicator is in essence with the carrying capacity of each strain when grown separate from the other. Given any two competing strains, we found that the winning strain almost always had the higher carrying capacity when grown in isolation. This in turn depends on the corresponding proportion of vegetative and heterocyst cells produced during reproduction. Given equal initial conditions for both compartments, the carrying capacity holds true as the winning factor in 97–98% of cases when the full parameter space $\mathbb{R}^{22}$ is sampled randomly (details in Supplementary Information). The 2–3% exceptions correspond to cases in which (i) a numerical error occurs; (ii) $p_v \simeq p_{v'}$, hence the time required for the populations to stabilize is longer than the simulation time; (iii) the p_v value of the loser is too close to 0, hence the corresponding population can not grow.

3.2.1. The optimal rate of differentiation (p_v)

The vegetative cell steady state is dependent on the p_v value. We found that the carrying capacity for the number of vegetative cells is a parabola-shaped function of p_v (Figs. 5a and b). Hence, a population too rich in vegetative cells would be disadvantaged in comparison to a population with p_v closer to the maximum of the curve. Identical optimality conditions hold in both models. Interestingly, the fact that the optimal p_v values are usually above 0.5 agrees with other theoretical work (Willensdorfer, 2009) indicating that the optimal fraction of germline cells in simple multicellular organisms will be higher than that of somatic cells. It also agrees with the high proportion of vegetatives seen in cyanobacteria. Figs. 5a and b illustrate further what we see in Figs. 4a and b. Consider the outcome of competitions between a pair of strains with different p_v values. In the single-celled model, the strain with the higher p_v wins, hence the optimal p_v can be surpassed (Fig. 5a). In the compartmental model, the population with the potential of a higher carrying capacity outcompetes the other, getting closer to the optimal p_v (Fig. 5b). These results led us to consider the case of repeated competitions, as analyzed in the following section.

3.2.2. Evolutionary optimization of p_v

Stochastic simulations of successive mutational events test the ability of the two strategies to evolve towards the optimal p_v value. Fig. 5c shows the outcome in the single-celled model. The population always evolves to a full cheater situation, where $p_v = 1$. Hence in this case optimization is not possible. After each mutational event the population with the higher p_v value will go to fixation, hence increasing p_v towards 1. Fig. 5d shows the results of the compartmental model. In this case, mutant competitions automatically lead to optimization towards the p_v value that corresponds to the maximum steady state value of vegetatives. Hence, compartmentalization allows populations to evolve towards optimal ratios of vegetative to heterocyst cells.

3.3. Duration of daylight and separation of tasks in time and space

We numerically solve systems (5) and (10) separately. Fig. 6b shows the steady states of the populations going from permanent darkness to permanent light. In the extreme case of permanent darkness, both species die because of the lack of photosynthesis. In regimes where the percentage of daylight is scarce, the species following the external day/night periodicity reaches a higher carrying capacity than the multicellular differentiated species. The opposite happens when the duration of daylight is much longer

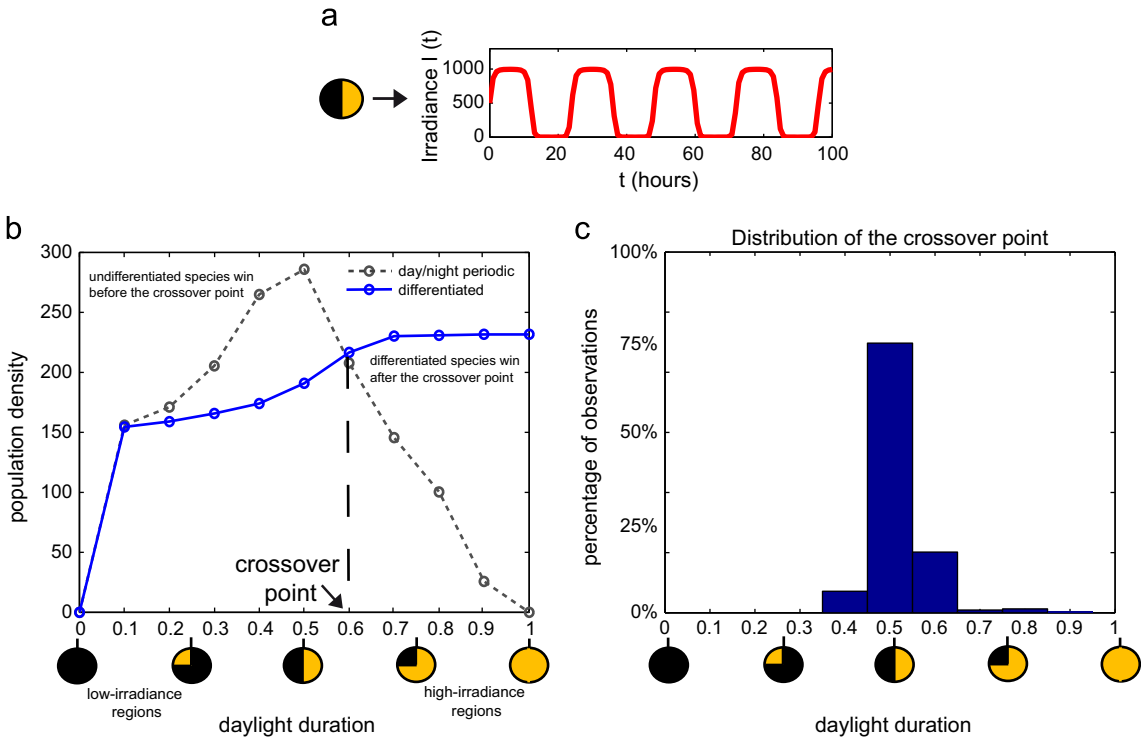

Fig. 6. (a) The irradiance function $I(t)$ as in Eq. (7), when the duration of day and night are equal. (b) Case study showing the comparison of the steady states of species following the day/night periodicity vs. differentiated species for different external irradiance cycles. Undifferentiated species that follow the day/night alternation reach a higher steady state in environments where the daylight is less or equal than the dark period. The point after which the differentiated species perform better is indicated by the crossover point. (c) Distribution of the crossover points based on 6000 trials. Parameters a, q, r, A were sampled randomly. In 75% of the cases, the crossover point locates around 0.5.

than night. In the latter case, as the daylight period is extended, the production of nitrogen decreases in the undifferentiated bacteria until it equals or is less than its consumption. In this situation the steady state population decays to 0. Hence long daylight conveys an advantage to division of tasks in space by means of differentiation. Fig. 6b shows a crossover point between the steady states, after which periodic species perform worse than the heterocystous. In order to check the occurrence and the location of a crossover point for a more general parameter space, we repeated 6000 times the scan of external daylight percentage for the two models. Each time we sampled randomly the values of parameters a, q, r, A. We recorded the position of the crossover point and we plotted the corresponding distribution through the histogram showed in Fig. 6c. We can conclude that in the 98.8% of the cases, there was always a crossover point after which the undifferentiated species reach a lower carrying capacity than the differentiated species. In the majority of the cases (75% of the cases), the crossover point is at 0.5, corresponding to a situation where the duration of night equals that of the day. These results indicate that environments where the dark period is significantly longer than the daylight period can be disadvantageous to terminally differentiated species.

3.4. Phylogenetic relationships among cyanobacteria

Phylogenetic relationships from 16S rRNA sequence of 37 strains of cyanobacteria are shown in Fig. 7. Cyanobacterial species were grouped into classes I–V as described by Rippka et al. (1979). Only the Bayesian tree is shown. Though the topology is based on

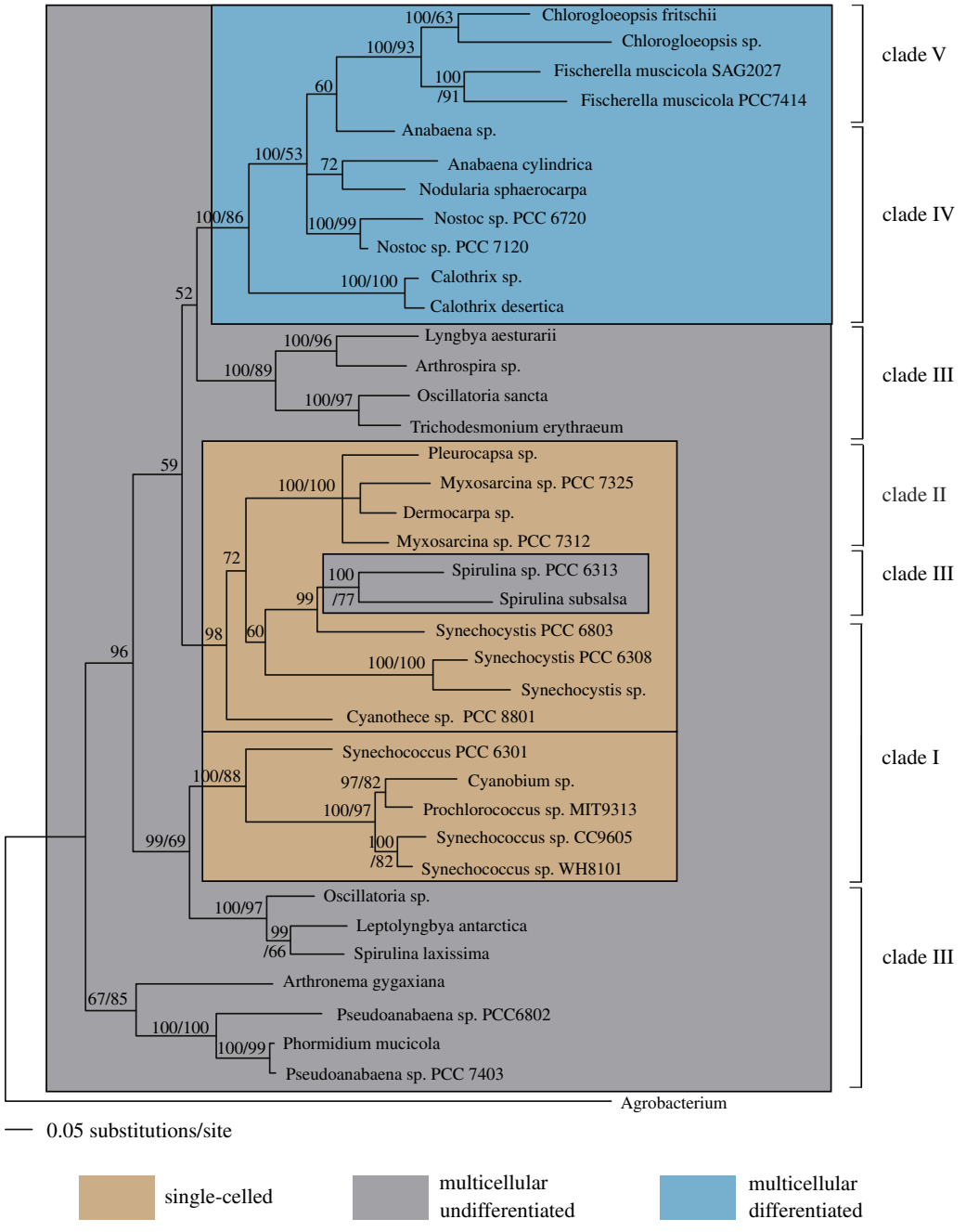

Fig. 7. Phylogenetic tree of cyanobacteria. Bayesian-tree of 16S rRNA sequences, based on GTR+I+G substitution model, with *Agrobacterium tumefaciens* as an outgroup. Shown at the nodes are only posterior probabilities (> 50%) or both posterior probabilities/bootstrap (> 50%). Posterior probabilities were calculated from 12,001 trees. Bootstrap values are obtained from 400 pseudo-replicates with maximum likelihood.

Bayesian analysis, character states are colored according to parsimony criteria (such that the least changes occur along the branches). Our analyses confirm the polyphyly of single celled clade I and the multicellular clade III as reported earlier (Giovannoni et al., 1988; Seo and Yokota, 2003). The multicellular, terminally differentiated clades IV and V, together form a monophyletic group supported by posterior probability (100%) and bootstrap (86%). The latter monophyly has been reported by other studies (Turner et al., 1999; Garcia-Pichel et al., 2001; Tomitani et al., 2006). Species from the polyphyletic clade III, belonging to the genera *Lyngbya*, *Arthrospira*, *Oscillatoria* and *Trichodesmium*, form the sister group of the monophyletic clade IV and V (blue box in Fig. 7). The phylogeny supports the conclusions of the simulations, according to which undifferentiated multicellularity evolved first, and hence made terminal differentiation possible.

4. Discussion

At first glance, multicellularity can appear as an obvious prerequisite for cellular differentiation. However, from a logical perspective, alternative developmental strategies are in principle possible. It has been recently emphasized (Leimar and Hammerstein, 2006) that it is important to strengthen the connection between theoretical models on the evolution of cooperation and explicit empirical cases. The framework we present here is formulated with this goal in mind, whereby we take a mechanistic representation of known biochemical interactions in an important group of organisms (the cyanobacteria), and show how the latter interactions fit into theoretical frameworks that attempt to explain multicellularity and the division of labor.

As discussed in Section 1.3, practically all solutions for avoiding the tragedy of the commons involve somehow separating the population into competing subsets. The results in Sections 3.1 and 3.2 are no exception to the latter rule. Compartmentalization allows for the protection of vital resources from potential disruptive mutations, whose effect can be limited to the compartment they arise in. Furthermore, multicellularity guarantees that cells in a compartment are clones. One could in fact make the argument that the evolution of multicellularity—and thereby compartmentalization—is a mechanistic means by which kin selection becomes "hard-wired" for a population of cooperating cyanobacterial cells. The cell interactions in the cyanobacterial system also have some structural similarities to a two component hypercycle with a single self-replicating catalyst, the main difference being the lack of hypergeometric growth in the bacterial case. It is noteworthy that conclusions previously derived from the study of hypercycles (Eigen and Schuster, 1978; Michod, 1983; Szathmáry and Demeter, 1987) can also apply to cell interactions and the evolution of multicellularity, indicating the potential generality of the former theory.

The cyanobacterial cell system has also some commonalities with cooperation games. The tragedy of the commons can for example be characterized by games like Prisoner's Dilemma, where the optimal strategy corresponds to cooperation of both players. However, the cell interactions that we consider do not directly map to a simple n-player game with a payoff matrix. Nonetheless, one may say that populations that converge on the optimal p_v, are in a state where all individuals are cooperating.

As seen in Section 3.2, the vegetative/heterocyst ratio has an effect on the carrying capacity of the population. Hence the tuning of the proportion of vegetatives upon division (p_v) can lead to the maximization of the carrying capacity. The autonomous optimization of the carrying capacity after repeated mutational events is found to be very different in the two models, as shown in Section 3.2.2. The single-celled strategy causes the fixation of the variant producing the most vegetative cells, thus converging to the value corresponding to the pure cheater case. Hence, higher fertility in the short term leads to decrease of carrying capacity and eventual extinction of the population in the long term. This explains why this evolutionary step is not observed in nature (see Fig. 8). On the other hand, the multicellular strategy allows for optimization towards the most profitable proportion of vegetative and heterocyst cells and for the selection and fixation of mutants that correspond to the maximal carrying capacity achievable by the population. Interestingly, this observation agrees with the almost constant vegetative/heterocysts ratio seen in many species of filamentous cyanobacteria (Adams, 2000).

The results from Sections 3.1 and 3.2 exclude the possibility of a transition from the undifferentiated unicellular stage to a differentiated single-celled one. Therefore, to achieve division of labor in cyanobacteria, two other paths are in principle possible: from undifferentiated unicellularity directly to differentiated multicellularity, or via the intermediate step of undifferentiated multicellularity (Fig. 8). The outcome of the phylogenetic analyses in Section 3.4 supports the second alternative, providing empirical evidence that the route to division of labor has included undifferentiated multicellularity. The combination of theoretical and phylogenetic results presented here lead to the conclusion that for the class of interactions occurring in cyanobacteria, multicellularity is a necessary condition for the evolution of terminal differentiation and the optimization of division of labor.

To further understand the ecological factors affecting the evolution of differentiation, we compared the advantages of a spatial separation of tasks over a temporal separation. The geographic distribution of cyanobacteria varies from mild to extreme environments (Paerl et al., 2000). It is known that environmental factors such as temperature can favor different forms of differentiation in cyanobacteria (Staal et al., 2003). However, there is at present no clear understanding of the distribution pattern of differentiated vs. undifferentiated cyanobacteria. According to results from our model of bacteria following the day/night periodicity in Section 3.3, division of labor by means of terminal differentiation is advantageous when the proportion of day is higher than that of night. In the latter case, the undifferentiated cyanobacteria fix nitrogen only in the short dark period. Meanwhile heterocystous cyanobacteria fix nitrogen also during the long day period. Conversely, in cases with scarce daylight, undifferentiated species have an advantage because during the short daylight period all available cells are devoted to light harvesting.

The undifferentiated species that we model simply follow the day/night alternation imposed by the external conditions, without the possibility of development of an internal cycle. The evolution of a self-regulated cycle that is independent of external light

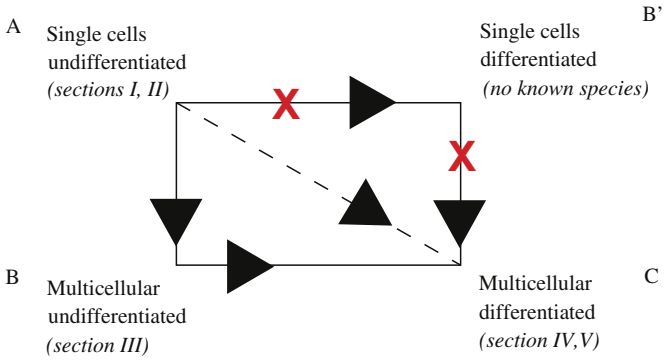

Fig. 8. The evolutionary paths leading to division of labor in cyanobacteria. A direct transition from A to C is in principle possible. Simulations exclude node B′. The phylogenetic tree supports the path B→C.

periodicity can potentially enhance the fitness of such bacteria, because the cycle can then be optimized according to the resource requirements. Hence if the circadian rhythm is optimized, there is the possibility that the undifferentiated circadian species can be also competitive in regions with long daylight periods. A true circadian rhythm has been observed in unicellular cyanobacteria such as *Synechococcus* (Mitsui et al., 1986). Further investigation of the benefits provided by an internal cycle could give an explanation for the maintenance of circadian rhythms in cyanobacteria. On the other hand, the development and regulation of such complex mechanisms is costly for the organism, and one may argue that a high cost of switching could support selection for differentiated species.

Our results on the response to daylight periodicities provide the general conclusion that in an environment with a short light period, selection acts against heterocystous cyanobacteria. In regimes of prevailing darkness, the absence of differentiation and the evolution of a circadian rhythm—or at least the simple adjustment according to the external periodicity—is advantageous. Hence adaptation to long daylight periods can be indicated as a possible reason for the evolution of terminal differentiation in cyanobacteria. The latter is a hypothesis that can be subject to empirical testing. Laboratory experiments can determine the outcome of competitions between undifferentiated and differentiated species under different day/night regimes. In addition, in order to determine seasonal differences between differentiated and undifferentiated species, ecological observations involving sample collection and quantitative measurements of species abundances could be systematically done in different seasons and at different latitudes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.jtbi.2009.09.009

References

Ackermann, M., Stecher, B., Freed, N.E., Songhet, P., Hardt, W.-D., Doebeli, M., 2008. Self-destructive cooperation mediated by phenotypic noise. Nature 454 (7207), 987–990.

Adams, D.G., 2000. Heterocyst formation in cyanobacteria. Current Opinion in Microbiology 3 (6), 618–624.

Agawin, N.S.R., Rabouille, S., Veldhuis, M.J.W., Servatius, L., Hol, S., van Overzee, H.M.J., Huisman, J., 2007. Competition and facilitation between unicellular nitrogen-fixing cyanobacteria and non-nitrogen-fixing phytoplankton species. Limnology and Oceanography 52 (5), 2233–2248.

Attolini, C.S.O., Stadler, P.F., 2006. Evolving towards the hypercycle: a spatial model of molecular evolution. Physica D—Nonlinear Phenomena 217 (2), 134–141.

Axelrod, R., Hamilton, W.D., 1981. The evolution of cooperation. Science 211 (4489), 1390–1396.

Bach, L.A., Helvik, T., Christiansen, F.B., 2006. The evolution of n-player cooperation—threshold games and ESS bifurcations. Journal of Theoretical Biology 238, 426.

Bergman, B., Gallon, J.R., Rai, A.N., Stal, L.J., 1997. N-2 fixation by non-heterocystous cyanobacteria. FEMS Microbiology Reviews 19 (3), 139–185.

Boerlijst, M.C., Hogeweg, P., 1991. Spiral wave structure in pre-biotic evolution—hypercycles stable against parasites. Physica D 48 (1), 17–28.

Bonner, J.T., 2000. First Signals: The Evolution of Multicellular Development. Princeton University Press, Princeton.

Boomsma, J.J., Franks, N.R., 2006. Social insects: from selfish genes to self organisation and beyond. Trends in Ecology and Evolution 21 (6), 303–308.

Brockhurst, M.A., Buckling, A., Racey, D., Gardner, A., 2008. Resource supply and the evolution of public-goods cooperation in bacteria. BMC Biology 6.

Buss, L.W., 1988. The Evolution of Individuality. Princeton University Press, Princeton, NJ.

Buss, L.W., 1982. Somatic-cell parasitism and the evolution of somatic tissue compatibility. Proceedings of the National Academy of Sciences of the United States of America—Biological Sciences 79 (17), 5337–5341.

Buss, L.W., 1983. Evolution, development, and the units of selection. Proceedings of the National Academy of Sciences of the United States of America—Biological Sciences 80 (5).

Doebeli, M., Knowlton, N., 1998. The evolution of interspecific mutualisms. Proceedings of the National Academy of Sciences 95 (15), 8676–8680.

Eigen, M., 1971. Selforganization of matter and evolution of biological macromolecules. Naturwissenschaften 58 (10), 465.

Eigen, M., Gardiner, W.C., Schuster, P., 1980. Hypercycles and compartments—compartments assists—but do not replace—hypercyclic organization of early genetic information. Journal of Theoretical Biology 85 (3), 407–411.

Eigen, M., Schuster, P., 1977. Hypercycle—principle of natural self-organization. A. emergence of hypercycle. Naturwissenschaften 64 (11), 541–565.

Eigen, M., Schuster, P., 1978. Hypercycle—principle of natural self-organization. B. abstract hypercycle. Naturwissenschaften 65 (1), 7–41.

Enquist, M., Leimar, O., 1993. The evolution of cooperation in mobile organisms. Animal Behaviour 45 (4), 747–757.

Ferriere, R., Bronstein, J.L., Rinaldi, S., Law, R., Gauduchon, M., 2002. Cheating and the evolutionary stability of mutualisms. Proceedings of the Royal Society of London B 296, 773–780.

Ferriere, R., Michod, R.E., 1996. The evolution of cooperation in spatially heterogeneous populations. American Naturalist 147 (5), 692–717.

Flores, E., Herrero, A., Wolk, C.P., Maldener, I., 2006. Is the periplasm continuous in filamentous multicellular cyanobacteria?. Trends in Microbiology 14 (10), 439–443.

Fontanari, J.F., Santos, M., Szathmary, E., 2006. Coexistence and error propagation in pre-biotic vesicle models: a group selection approach. Journal of Theoretical Biology 239 (2), 247–256.

Frank, S.A., 1994. Kin selection and virulence in the evolution of protocells and parasites. Proceedings of the Royal Society of London Series B—Biological Sciences 258 (1352), 153–161.

Garcia-Pichel, F., Lopez-Cortes, A., Nuebel, U., 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. Applied and Environmental Microbiology 67 (4), 1902–1910.

Gilbert, O.M., Foster, K.R., Mehdiabadi, N.J., Strassmann, J.E., Queller, D.C., 2007. High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. Proceedings of the National Academy of Science of the United States of America 104 (21), 8913–8917.

Giovannoni, S.J., Turner, S., Olsen, G.J., Barns, S., Lane, D.J., Pace, N.R., 1988. Evolutionary relationships among cyanobacteria and green chloroplasts. Journal of Bacteriology 170 (8), 3584–3592.

Griffin, A.S., West, S.A., Buckling, A., 2004. Cooperation and competition in pathogenic bacteria. Nature 430 (7003), 1024–1027.

Hamilton, W.D., 1964a. The genetical evolution of social behaviour. I. Journal of Theoretical Biology 7 (1), 1.

Hamilton, W.D., 1964b. The genetical evolution of social behaviour. II. Journal of Theoretical Biology 7 (1), 17.

Hammerstein, P., 2003. Why is reciprocity so rare in social animals? A protestant appeal. In: Hammerstein, P. (Ed.), Genetic and Cultural Evolution of Cooperation, Dahlem Workshop Reports: Environmental Sciences Research Report, 90th Dahlem Workshop on Genetic and Cultural Evolution of Cooperation, Berlin, Germany, June 23–28, 2002, pp. 83–93.

Han, B.P., 2001. Photosynthesis-irradiance response at physiological level: a mechanistic model. Journal of Theoretical Biology 213 (2), 121.

Hardin, G., 1968. The tragedy of the commons. Science 162, 143.

Hochberg, M., Rankin, D., Taborsky, M., 2008. The coevolution of cooperation and dispersal in social groups and its implications for the emergence of multicellularity. BMC Evolutionary Biology 8 (1), 238.

Hofbauer, J., Schuster, P., Sigmund, K., 1979. Evolutionary stable strategies and game dynamics. Journal of Theoretical Biology 81 (3), 609–612.

Hofbauer, J., Sigmund, K., 1998. Evolutionary Games and Population Dynamics. Cambridge University Press, Cambridge.

Hogeweg, P., Takeuchi, N., 2003. Multilevel selection in models of prebiotic evolution: compartments and spatial self-organization. Origins of Life and Evolution of Biospheres 33 (4), 375.

Honda, D., Yokota, A., Sugiyama, J., 1999. Detection of seven major evolutionary lineages in cyanobacteria based on the 16s rRNA gene sequence analysis with new sequences of five marine Synechococcus strains. Journal of Molecular Evolution 48 (6), 723–739.

Kageyama, H., Nishiwaki, T., Nakajima, M., Iwasaki, H., Oyama, T., Kondo, T., 2006. Cyanobacterial circadian pacemaker: Kai protein complex dynamics in the KaiC phosphorylation cycle in vitro. Molecular Cell 23 (2), 161–171.

Kaiser, D., 2001. Building a multicellular organism. Annual Review of Genetics 35 (1), 103–123.

Killingback, T., Bieri, J., Flatt, T., 2006. Evolution in group-structured populations can resolve the tragedy of the commons. Proceedings of the Royal Society B—Biological Sciences 273 (1593), 1477–1481.
Kondo, T., Strayer, C.A., Kulkarni, R.D., Taylor, W., Ishiura, M., Golden, S.S., Johnson, C.H., 1993. Circadian rhythms in prokaryotes—luciferase as a reporter of circadian gene expression in cyanobacteria. Proceedings of the National Academy of Sciences of the United States of America 90 (12), 5672–5676.
Kurosawa, G., Aihara, K., Iwasa, Y., 2006. A model for the circadian rhythm of cyanobacteria that maintains oscillation without gene expression. Biophysical Journal 91 (6), 2015–2023.
Lawlor, D.W., 1990. Photosynthese. Georg Thieme Verlag, Stuttgart.
Lehmann, L., Keller, L., 2006. The evolution of cooperation and altruism—a general framework and a classification of models. Journal of Evolutionary Biology 19 (5), 1365–1376.
Leimar, O., Hammerstein, P., 2001. Evolution of cooperation through indirect reciprocity. Proceedings of the Royal Society of London Series B—Biological Sciences 268 (1468), 745–753.
Leimar, O., Hammerstein, P., 2006. Facing the facts. Journal of Evolutionary Biology 19 (5), 1403–1405.
Mariscal, V., Herrero, A., Flores, E., 2007. Continuous periplasm in a filamentous heterocyst-forming cyanobacterium. Molecular Microbiology 65 (4), 1139–1145.
Marler, M.R., Gehrman, P., Martin, J.L., Ancoli-Israel, S., 2006. The sigmoidally transformed cosine curve: a mathematical model for circadian rhythms with symmetric non-sinusoidal shapes. Statistics in Medicine 25 (22), 3893–3904.
Michod, R.E., 2007. Evolution of individuality during the transition from unicellular to multicellular life. Proceedings of the National Academy of Sciences 104 (Suppl. 1), 8613–8618.
Michod, R.E., 1983. Population biology of the 1st replicators—on the origin of the genotype, phenotype and organism. American Zoologist 23 (1), 5–14.
Michod, R.E., Roze, D., 2001. Cooperation and conflict in the evolution of multicellularity. Heredity 86 (Part 1), 1–7.
Michod, R.E., Viossat, Y., Solari, C.A., Hurand, M., Nedelcu, A.M., 2006. Life-history evolution and the origin of multicellularity. Journal of Theoretical Biology 239.
Mitsui, A., Kumazawa, S., Takahashi, A., Ikemoto, H., Cao, S., Arai, T., 1986. Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. Nature 323 (6090), 720–722.
Mullineaux, C.W., Mariscal, V., Nenninger, A., Khanum, H., Herrero, A., Flores, E., Adams, D.G., 2008. Mechanism of intercellular molecular exchange in heterocyst-forming cyanobacteria. EMBO Journal 27 (9), 1299–1308.
Nakamaru, M., Matsuda, H., Iwasa, Y., 1997. The evolution of cooperation in a lattice-structured population. Journal of Theoretical Biology 184 (1), 65–81.
Nowak, M.A., Bonhoeffer, S., May, R.M., 1994. Spatial games and the maintenance of cooperation. Proceedings of the National Academy of Sciences of the United States of America 91 (11), 4877–4881.
Nowak, M.A., May, R.M., 1992. Evolutionary games and spatial chaos. Nature 359 (6398), 826–829.
Ohtsuki, H., Hauert, C., Lieberman, E., Nowak, M.A., 2006. A simple rule for the evolution of cooperation on graphs and social networks. Nature 441 (7092), 502–505.
Paerl, H.W., Pinckney, J.L., Steppe, T.F., 2000. Cyanobacterial-bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. Environmental Microbiology 2 (1), 11–26.
Partensky, F., Hess, W.R., Vaulot, D., 1999. Prochlorococcus, a marine photosynthetic prokaryote of global significance. Microbiology and Molecular Biology Reviews 63 (1), 106–127.
Pfeiffer, T., Bonhoeffer, S., 2003. An evolutionary scenario for the transition to undifferentiated multicellularity. Proceedings of the National Academy of Sciences 100 (3), 1095–1098.
Pfeiffer, T., Schuster, S., Bonhoeffer, S., 2001. Cooperation and competition in the evolution of ATP-producing pathways. Science 292 (5516), 504–507.
Rabouille, S., Staal, M., Stal, L., Soetaert, K., 2006. Modeling the dynamic regulation of nitrogen fixation in the cyanobacterium Trichodesmium sp. Applied and Environmental Microbiology 72 (5), 3217–3227.
Rainey, P.B., Rainey, K., 2003. Evolution of cooperation and conflict in experimental bacterial populations. Nature 425 (6953), 72–74.
Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., Stanier, R.Y., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology 111, 1–61.
Roberts, G., Sherrat, T.N., 1998. Development of cooperative relationships through increasing investment. Nature 394, 175–179.
Roussel, M.R., Gonze, D., Goldbeter, A., 2000. Modeling the differential fitness of cyanobacterial strains whose circadian oscillators have different free-running periods: comparing the mutual inhibition and substrate depletion hypotheses. Journal of Theoretical Biology 205 (2), 321–340.
Rubio, F.C., Camacho, F.G., Sevilla, J.M.F., Chisti, Y., Grima, E.M., 2003. A mechanistic model of photosynthesis in microalgae. Biotechnology and Bioengineering 81 (4), 459–473.
Sachs, J.L., Mueller, U.G., Wilcox, T.P., Bull, J.J., 2004. The evolution of cooperation. The Quarterly Review of Biology 79 (2), 135–160.
Saier, M., Jacobson, G.R., 1984. The Molecular basis of Sex and Differentiation. Springer, New York.
Sardanyés, J., Solé, R.V., 2006. Bifurcations and phase transitions in spatially extended two-member hypercycles. Journal of Theoretical Biology 243 (4), 468–482.
Schleip, W., 1934. August Weismanns Bedeutung für die Entwicklung der Zoologie und allgemeinen Biologie zu seinem hundertsten Geburtstag am 17. Januar 1934. Naturwissenschaften 22 (3).
Schopf, J.W., 1994. Disparate rates, differing fates: tempo and mode of evolution changed from the Precambrian to the Phanerozoic. Proceedings of the National Academy of Sciences 91, 6735–6742.
Seo, P.S., Yokota, A., 2003. The phylogenetic relationships of cyanobacteria inferred from 16srRNA, gyrB, rpoC1 and rpoD1 gene sequences. The Journal of General and Applied Microbiology 49.
Shapiro, J.A., 1998. Thinking about bacterial populations as multicellular organisms. Annual Review of Microbiology 52 (1), 81–104.
Smith, J.M., 1964. Group selection and kin selection. Nature 201, 1145–1147.
Smith, J.M., 1982. Evolution and the Theory of Games. Cambridge University Press, Cambridge.
Smith, J.M., 1979. Hypercycles and the origin of life. Nature 280 (5722), 445–446.
Smith, J.M., Price, G.R., 1973. Logic of animal conflict. Nature 246 (5427), 15–18.
Smith, J.M., Szathmáry, E., 1995. The Major Transitions in Evolution. Freeman, Oxford.
Staal, M., Meysman, F.J.R., Stal, L.J., 2003. Temperature excludes N-2-fixing heterocystous cyanobacteria in the tropical oceans. Nature 425 (6957) 504–507.
Stal, L.J., Krumbein, W.E., 1987. Temporal separation of nitrogen-fixation and photosynthesis in the filamentous, nonheterocystous cyanobacterium Oscillatoria sp. Archives of Microbiology 149 (1), 76–80.
Strassmann, J.E., Zhu, Y., Queller, D.C., 2000. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. Nature 408 (6815), 965–967.
Suzuki, S., Akiyama, E., 2008. Chaos, oscillation and the evolution of indirect reciprocity in n-person games. Journal of Theoretical Biology 252, 686.
Szathmáry, E., Demeter, L., 1987. Group selection of early replicators and the origin of life. Journal of Theoretical Biology 128 (4), 463–486.
Tilman, D., 1977. Resource competition between planktonic algae—experimental and theoretical approach. Ecology 58 (2), 338–348.
Tomitani, A., Knoll, A.H., Cavanaugh, C.M., Ohno, T., 2006. The evolutionary diversification of cyanobacteria: molecular-phylogenetic and paleontological perspectives. Proceedings of the National Academy of Sciences of the United States of America 103 (14), 5442–5447.
Travisano, M., Velicer, G.J., 2004. Strategies of microbial cheater control. Trends in Microbiology 12 (2), 72–78.
Trivers, R.L., 1971. Evolution of reciprocal altruism. Quarterly Review of Biology 46 (1), 35.
Turner, S., Pryer, K.M., Miao, V.P.W., Palmer, J.D., 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small submit rRNA sequence analysis. Journal of Eukaryotic Microbiology 46 (4), 327–338.
Velicer, G.J., Kroos, L., Lenski, R.E., 2000. Developmental cheating in the social bacterium *Myxococcus xanthus*. Nature 404 (6778), 598.
Vrede, K., Heldal, M., Norland, S., Bratbak, G., 2002. Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. Applied and Environmental Microbiology 68 (6), 2965–2971.
Weismann, A., 1889. Essays Upon Heredity. Oxford At the Clarendon Press (Chapters II, IV) ⟨http://www.esp.org:80/books/weismann/essays/facsimile/⟩.
West, S.A., Griffin, A.S., Gardner, A., Diggle, S.P., 2006. Social evolution theory for microorganisms. Nature Reviews Microbiology 4 (8), 597–607.
Willensdorfer, M., 2009. On the evolution of differentiated multicellularity. Evolution 63 (2), 306–323.
Wolk, C.P., 1996. Heterocyst formation. Annual Review of Genetics 30, 59–78.