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Size evolution in microorganisms masks trade-offs predicted by the growth rate hypothesis

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Adaptation to local resource availability depends on responses in growth rate and nutrient acquisition. The growth rate hypothesis (GRH) suggests that growing fast should impair competitive abilities for phosphorus and nitrogen due to high demand for biosynthesis. However, in microorganisms, size influences both growth and uptake rates, which may mask trade-offs and instead generate a positive relationship between these traits (size hypothesis, SH). Here, we evolved a gradient of maximum growth rate (μ_{max}) from a single bacterium ancestor to test the relationship among μ_{max}, competitive ability for nutrients and cell size, while controlling for evolutionary history. We found a strong positive correlation between μ_{max} and competitive ability for phosphorus, associated with a trade-off between μ_{max} and cell size: strains selected for high μ_{max} were smaller and better competitors for phosphorus. Our results strongly support the SH, while the trade-offs expected under GRH were not apparent. Beyond plasticity, unicellular populations can respond rapidly to selection pressure through joint evolution of their size and maximum growth rate. Our study stresses that physiological links between these traits tightly shape the evolution of competitive strategies.

1. Introduction

Species persistence depends on their adaptation to local resource supply [1]. Two key components of this adaptation are how fast organisms grow and how efficient they are at using available resources. In addition, the evolution of an optimal competitive strategy is constrained by the physiological link between maximum growth rate and nutrient requirements [2,3]. In this study, we use experimental evolution to investigate the consequences of these interdependencies on the emergence of competitive strategies.

In a fluctuating environment, rapid growth can be the optimal strategy [4,5]. For instance, ruderal plants (*sensu* [6]) grow quickly and are efficient colonizers in habitats where disturbance prevents other species from establishing and depleting inorganic nutrients [7]. Similarly, where inorganic nutrient supply is high, fast-growing cyanobacteria or microalgae can create blooms by escaping predator

regulation [8,9]. However, the capacity for rapid growth implies that more resources are allocated to biosynthesis, potentially at the expense of other biological functions such as defence or resource acquisition (see [2] for a review and [10]). Therefore, optimizing the maximum *per capita* growth rate (hereafter called μ_{max}) might be detrimental to other traits, such as the ability to survive in low-resource conditions [11,12], or resistance to predation [13,14].

In a constant environment, consumer–resource theory predicts that the species that can maintain a viable population at the lowest resource concentration (R^*) will exclude all its competitors regardless of their growth rate [15,16]. Microcosm experiments have demonstrated competitive exclusion under nutrient limitation, for instance, among diatoms and unicellular algae [17,18] on a gradient where species were limited either by phosphorus (where diatoms win) or by silicon (where algae win). Hence, in a stable environment without disturbance to disrupt competitive exclusion, the most successful long-term strategy should be the one that minimizes R^*, hence maximizing what we hereafter call competitive ability for the resource C_R, with $C_R \propto 1/R^*$.

The trade-off between strategies that maximize the growth rate (μ_{max}) in fluctuating environments or competitive ability (C_R) in constant environments is usually referred to as the gleaner–opportunist trade-off [5,19,20]. More generally, it can be interpreted as part of the classical trade-off between r and K strategies [4].

Ecological stoichiometry stresses the importance of biochemical constraints in producing such trade-offs between traits [21]. The biological functions prioritized by a given strategy (for example, biosynthesis or resource acquisition) involve specific synthesis pathways, whose molecular demands will lead to different nutrient requirements at the organismal scale [10]. For instance, the growth rate hypothesis (GRH) [22] states that growing faster requires greater amounts of RNA to sustain biosynthesis [23–25]. As nucleic acids are rich in phosphorus and to a lesser extent in nitrogen, this results in a higher demand for phosphorus in fast-growing organisms than in slow-growing ones [21,26,27]. Some experiments have shown that phosphorus limitation reduces both the growth rate and amount of intracellular RNA [28]. The logical corollary would be that fast-growing species should be less tolerant to phosphorus deficiency than slow-growing ones. In particular, they should be less competitive in constant environments where phosphorus is the limiting nutrient [11]. As a consequence, a negative relationship between growth rate and competitive ability for phosphorus is predicted by the GRH (figure 1). As nucleic acids are also moderately richer in nitrogen than other cellular molecules, a similar but weaker trend should hold for nitrogen.

For microorganisms, however, variation in cell size may make it difficult to observe such a trade-off. Small organisms may grow faster than large ones (e.g. picoplankton: [29]), possibly due to their shorter replication process and cell division time [30–32]. In addition, when diffusion is the limiting factor in acquiring nutrients, smaller osmotrophs with their high surface-to-volume ratios are expected to be more efficient than larger ones in acquiring the nutrients required for their growth [29,33]. This negative correlation between cell size and nutrient affinity has been found in diverse taxa of phytoplankton and bacteria [29,34,35]. If cell size is negatively related to both competitive ability for nutrients (C_R) and maximum growth rate (μ_{max}), the variability of
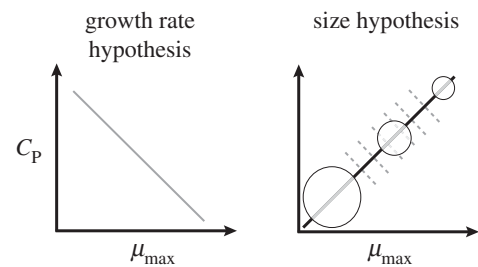

Figure 1. Hypotheses on the expected relationship among maximum growth rate, μ_{max} and competitive ability for phosphorus, C_P. The GRH predicts a negative relationship (grey lines), whereas the SH predicts that variability in organism sizes (circles) masks the GRH's predicted trade-off which may still occur for a given cell size (grey dotted lines) and produces a positive relationship (black line): small cells exhibit both high μ_{max} and high C_P.

organisms' size may generate a positive correlation between these two traits, hereafter referred to as the size hypothesis (SH; figure 1).

Overall, both nutrient availability and physiology constrain the evolution of the above-mentioned traits (C_R, μ_{max} and cell size) and the emergence of competitive strategies in microorganisms. The relationships between these traits have been studied theoretically and observed in cross-taxon comparisons [34–36]. Comparative analysis, however, has limited utility for inference due to lack of independence between closely related taxa. Sister species are more likely to have similar trait values according to a Brownian model of trait evolution. Therefore, trade-offs among traits may emerge simply due to the constraint of evolutionary history, depending on the identity and relatedness of species compared.

Our objective was to investigate the relationship between the capacity for rapid growth (μ_{max}), competitive ability for nutrients (C_P and C_N for phosphorus and nitrogen, respectively), and cell size using experimental evolution of microbial cells. We took advantage of the rapid multiplication rate of bacteria to study how physiology constrains the joint evolution of these traits. Starting with a single ancestral strain allowed us to control for unknown evolutionary history, rendering a phylogenetic regression unnecessary. We analysed the emergence of competitive strategies in the bacterium *Pseudomonas fluorescens* SBW25 from a single clone selected at different phases of a growth period (growing phase versus stationary phase). We expected these treatments to select different maximum *per capita* growth rates μ_{max}. We then examined how evolutionary pressure leading to the diversification of μ_{max} also affected cell size and competitive ability for nutrients. Hence we tested whether the relationships between traits in our evolved strains support the GRH, the SH or a combination of both: according to the GRH, fast-growing bacteria should display a low competitive ability for phosphorus (and to a lower extent for nitrogen) compared to slow-growing bacteria, whereas we expect the reverse with the SH (figure 1).

2. Material and methods

We experimentally evolved strains from a single bacterial clone of *P. fluorescens* SBW25 in batch cultures where populations were selected at different phases of the growth period (figure 2). We then measured the maximum *per capita* growth rate, μ_{max}, and competitive ability for phosphorus (P) and nitrogen (N), C_P and C_N, respectively, of the evolved and ancestral strains, from
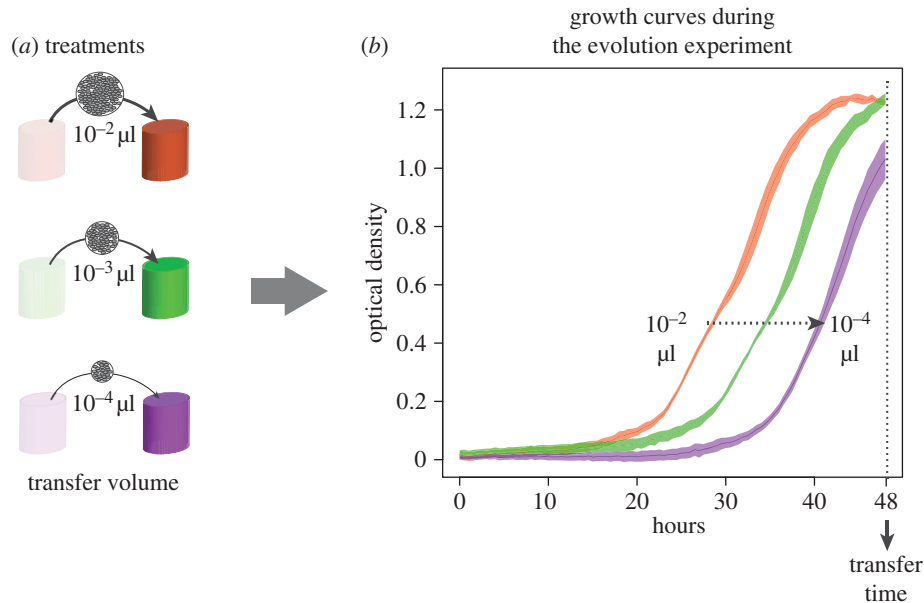
Figure 2. Experimental evolution design (*a*) and growth curves of strains during experimental evolution (*b*). Bacteria populations were transferred every 48 h to new medium. Treatments consisted of varying the transfer volume (TV) from 10^{-2} to 10^{-4} μl, which corresponds to a decreasing number of bacteria transferred (*a*). These initial differences, though not detectable by optical density, led to delayed growth between treatments after few transfers, here at day 12 as an example (*b*). Lines represent average values over six evolution replicates (strains) by treatment. Coloured areas represent standard deviation. (Online version in colour.)

growth curves realized on either low-P or low-N media. We estimated relative cell sizes for each strain by flow cytometry, and their nutrient relative content in the cell (q_P and q_N quotas), hereafter called content, by X-rays (required to estimate C_P and C_N). We finally analysed the correlations among these estimators of cell size, μ_{max}, C_P and C_N.

(a) Evolution experiment

We started cultures from a single isogenic population of *P. fluorescens* SBW25 mutS- (provided by Escobar-Páramo *et al.* [37]) to standardize the initial trait variability and evolutionary history. We randomly chose six clonal colonies from this population to form our six ancestral populations. We grew bacteria in a medium designed to independently vary nitrogen and phosphorus concentrations in later assays (see the electronic supplementary material for full methods). We evolved strains from these six ancestors for two months (approx. 300 generations) in batch culture: a small proportion of each 200 μl culture was transferred every 48 h into new medium (figure 2*a*) in order to maintain bacterial growth. Selection treatments were implemented by varying the volume of culture transferred (TV) from 10^{-2} to 10^{-4} μl (hereafter referred to as TV10^{-2}, TV10^{-3} and TV10^{-4}). Initial densities for each 48 h growth period (between two transfers) increased with the TV, thus allowing bacteria to reach the stationary phase more quickly before the next transfer when more cells were transferred. The range of TV therefore enabled us to evolve strains on a continuum between the growing and stationary phases (figure 2*b*). Bacteria selected in the growing phase evolved at low population density and high resource availability, whereas populations selected in the stationary phase experienced high population density and low-resource availability.

(b) Trait measurements

After the evolution experiment, we measured (i) the content of phosphorus and nitrogen (q_P and q_N), (ii) the competitive ability for phosphorus and nitrogen (C_P and C_N), (iii) the maximum *per capita* growth rate μ_{max}, and (iv) the relative cell size of the strains:

(i) For each of the evolved strains we measured the relative elemental content of 10 cells by X-ray (electronic supplementary material, section *Stoichiometry*). We estimated

the phosphorus and nitrogen content by using the average ratio of the respective element to the sum of carbon and oxygen content, because carbon and oxygen represent most cell biomass [21].

(ii) For a given resource, the competitive ability was estimated by $1/R^*$. The R^* is the minimum resource level required for a population to sustain positive biomass, and also represents the surplus resource left in the environment when the population has reached equilibrium. Given that $R^* = T_R - B^*q_R$, and that the total amount of resource T_R is held constant in our experiment, the R^* is proportional to the biomass at equilibrium (B^*) multiplied by its resource content (q_R). The data on stoichiometry (i) showed that q_N and q_P were independent of the maximum growth rate μ_{max} (table 1; electronic supplementary material, figure S1), and thus would not bias the relationship of interest between μ_{max} and R^*. Knowing that, we considered the other part of our estimator of R^*, the biomass at equilibrium B^*, to explain the potential correlation between competitive ability for nutrients (C_P or C_N) and μ_{max}. Subsequently, we estimated C_P and C_N as the biomass at equilibrium when the nutrient, N or P, was limiting (determined by preliminary assays). We recorded growth continuously for 70 h on medium where either P was diluted by 60 (hereafter called low-P medium; N : P of 16.09 : 1) or N diluted by 3 (hereafter called low-N medium; N : P of 0.09 : 1), compared to the medium the strains had evolved on (N : P of 0.27 : 1). We grew five replicate populations for each of the 24 strains (ancestral + evolved strains) on each medium. The biomass at the plateau of the growth curve, in this case the maximum optical density measured by spectrophotometry at 650 nm, was used to estimate the equilibrium biomass B^* on a given limiting resource (N or P). Optical density allows integration of variations in cell shape, size and density in the biomass measurement [38]. This biomass was the estimator of the competitive ability for this resource, C_P or C_N.

(iii) We estimated the maximum *per capita* growth rate μ_{max} from the same growth curves. We computed μ_{max} as the maximum gain of biomass per hour and biomass unit (optical density). This method provided more robust

Table 1. Tests of evolution treatment effects on traits. Kruskal−Wallis non-parametric tests on ranks for all traits except cell size, for which homogeneous variances allowed ANOVA testing. Letters refer to significantly different means among treatments according to post-hoc multiple comparisons (with a > b > c), using non-parametric tests for all traits except cell size, which used Tukey's HSD test. Treatments abbreviated as follows: ancestors (A), TV10^{-2} (T2), TV10^{-3} (T3), TV10^{-4} (T4).

| | | | | | multiple comparisons | | | |
data	n	d.f.	statistics	p-value	A	T2	T3	T4
$\mu_{max}^{(low\text{-}P)} \sim$ treatments	24	3	$\chi^2 = 16.63$	$<0.001^{**}$	a	c	bc	ab
$C_P \sim$ treatments	24	3	$\chi^2 = 17.64$	$<0.001^{**}$	ab	a	ab	b
$\mu_{max}^{(low\text{-}N)} \sim$ treatments	24	3	$\chi^2 = 12.78$	$<0.010^{*}$	a	b	b	ab
$C_N \sim$ treatments	24	3	$\chi^2 = 1.77$	0.621				
cell size $\sim$ treatments	24	3	$F = 24.03$	$<0.0001^{***}$	b	a	b	c
$q_P \sim$ treatments (without A)	18	2	$\chi^2 = 1.17$	0.557				
$q_N \sim$ treatments (without A)	18	2	$\chi^2 = 1.20$	0.548				

results than fitting an exponential curve, because of the low precision of biomass estimates at low density, and delays in growth response. We thus obtained different growth rates on low-P and low-N media, $\mu_{max}^{(low\text{-}P)}$ and $\mu_{max}^{(low\text{-}N)}$, respectively, which were positively correlated (Spearman's rank correlation: $\rho = 0.65$, $n = 18$, $p < 0.01$; electronic supplementary material, figure S2).

(iv) Finally, we assessed relative cell sizes by flow cytometry (cytometer FACSCalibur^{TM}). We grew six replicate populations in King's broth medium for each of the 18 evolved strains and six ancestral strains for 18 h. We checked that all strains had passed the exponential phase, so that the cells sampled covered a wide range of physiological stages, rather than sampling only small cells experiencing division. We recorded the FSC-H parameter, which was previously shown to be associated with cell size [39–41], is commonly used for qualitative comparisons of bacteria sizes (e.g. [38,42]), and provides distributions on a large number of cells (here 50 000 cytometer events per sample with a flow of 800–1200 events per second). We used the geometric mean of the FSC-H distribution as a proxy for a population's average cell size (relative proxy, not quantitative measurement). The geometric mean better captures the diversity of a population with large distributions than does arithmetic mean, because it is less sensitive to high values (however, the arithmetic means gave similar results in our case).

(c) Statistical analyses

We compared $\mu_{max}^{(low\text{-}N)}$, $\mu_{max}^{(low\text{-}P)}$, C_P, C_N, q_P and q_N between treatments using the non-parametric Kruskal−Wallis' test by ranks, and mean cell size with an ANOVA, to assess the effect of the treatments on traits. We used non-parametric tests when variances were heterogeneous according to Bartlett's test. We applied the tests on replicate means to avoid pseudo-replication. We then used post-hoc multiple comparison tests, with the function kruskalmc of the pgirmess R package 1.5.9 [43] for non-parametric tests (based on the methods in [44]) and Tukey's HSD test for parametric tests, to determine which treatments were significantly different from one another for each trait. We then calculated Spearman's rank correlation coefficients to characterize the relationships between traits.

3. Results

The evolution treatments resulted in an extended gradient of maximum growth rate μ_{max} on low-P and low-N media (figure 3a,b). Strains selected at the stationary phase with large transferred volumes (TV10^{-2}) had significantly lower μ_{max} compared with ancestral strains or strains selected at the growing phase in low-density conditions (TV10^{-4}; table 1 and figure 3). There were, however, no significant differences of μ_{max} between treatments on low-N medium (Kruskal−Wallis' test: $\chi^2_{2,18} = 3.40$, $p = 0.182$). In addition, the μ_{max} of all strains was lower when grown in low-P medium than when grown in low-N medium (means were significantly different: Mann−Whitney's test, $p < 0.0001$, and $\mu_{max}^{(low\text{-}P)}(0.27) < \mu_{max}^{(low\text{-}N)}(0.39)$). Evolution treatments also significantly affected the average cell size ($F_{3,20} = 24.03$; $p < 0.0001$; table 1 and figure 3c), with size increasing with higher transferred volume. When bacteria were selected at the stationary phase in high-density conditions, there was an increased number of large cells, even though small cells were still abundant, which resulted in larger average cell size compared with bacteria selected at the growing phase in low-density conditions (see cell size distributions in the electronic supplementary material, figure S3).

We found a positive correlation between maximum growth rate $\mu_{max}^{(low\text{-}P)}$ and competitive ability, C_P, when bacteria were maintained under phosphorus limitation (figure 4a; Spearman's test: $\rho = 0.76$, $n = 18$, $p < 0.001$). Strains selected at the growing phase in low-density conditions (TV10^{-4}) displayed greater μ_{max} and greater C_P than strains selected at the stationary phase in high-density conditions (table 1 and figure 4a). We also found a clear negative relationship between cell size and the ability to grow fast when phosphorus was limiting (Spearman's test: $\rho = -0.80$, $n = 18$, $p < 0.001$; figure 4b) and between cell size and C_P (Spearman's test: $\rho = -0.839$, $n = 18$, $p < 0.0001$; electronic supplementary material, figure S4a). By contrast, when bacteria were maintained under nitrogen limitation, there was no significant relationship between any of the measured traits (table 1; electronic supplementary material, figures S4b and S5).

4. Discussion

Selecting strains during the growing phase (small TV) resulted in 'fast-competitor' bacteria which displayed both high maximum growth rates μ_{max} and high competitive ability for phosphorus, C_P, but small cell sizes. By contrast, selecting strains during the stationary phase (large TV), resulted in larger
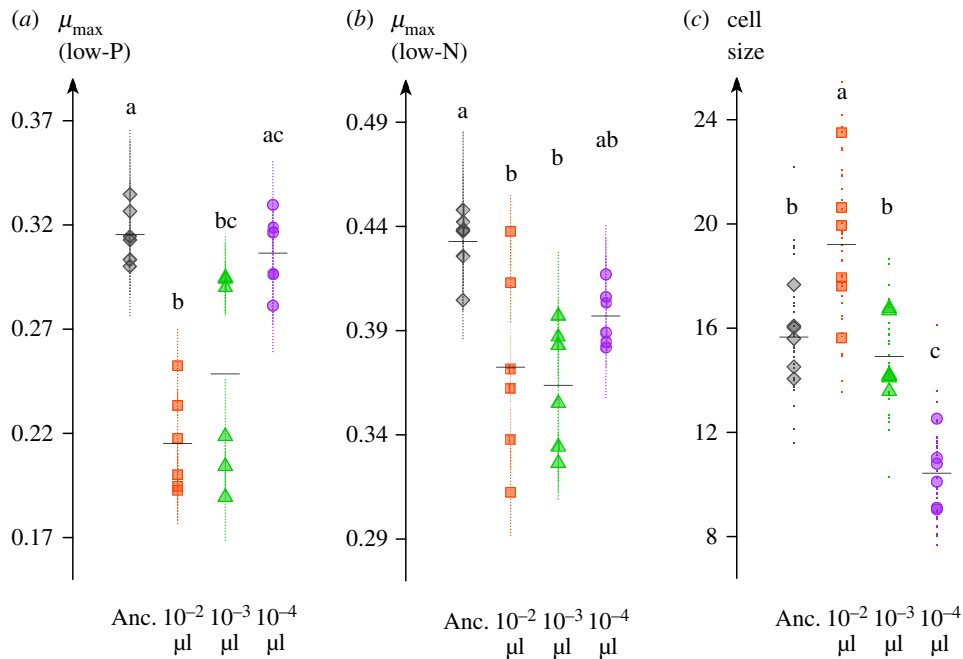
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Figure 3. Maximum growth rates, μ_{max}, on low-P (a) and low-N (b) media, and relative cell size (c) of ancestors (Anc.) and evolved strains (treatments = transferred volumes in microlitres). Relative cell size is estimated by the FSC-H parameter measured by flow cytometry over 50 000 events by population. Symbols represent averages of five measurements for each of the six strains within a treatment. Bars represent means. Letters refer to significantly different means according multiple comparisons, where such tests were appropriate (see table 1 for tests results). (Online version in colour.)
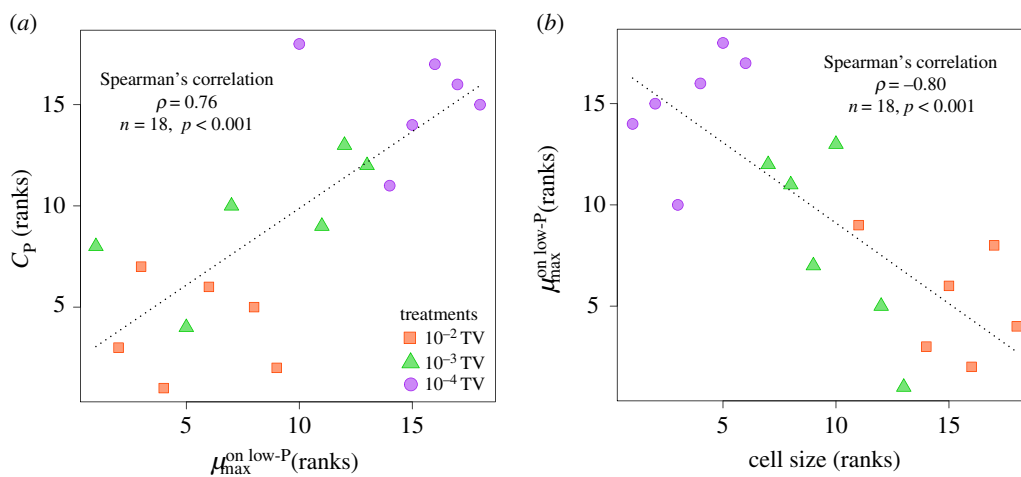

Figure 4. Relationship between maximum growth rate μ_{max} on low-P medium and competitive ability for Phosphorus C_P (a), or relative cell size (b), of evolved strains. Values were rank transformed to correspond to the non-parametric test we used. Competitive ability was approximated with maximum optical density obtained during 70 h of bacterial growth. Relative cell size was approximated with the geometric mean of the FSC-H parameter distribution of 50 000 events by population measured with flow cytometry. Symbols represent averages of five measurements per strain. TV units are in microlitres. (Online version in colour.)

average cell sizes. The maximum growth rate of such strains, as well as their competitive ability when phosphorus was limiting, were lower than those of bacteria selected during the growing phase. Surprisingly, we found no significant relationship when nitrogen was limiting.

(a) No growth rate hypothesis trade-off

The maximum growth rate μ_{max} of all strains decreased on phosphorus-limited medium (figure 3) compared with low-N medium where P was not limiting. This confirms the positive link between phosphorus requirements for biosynthesis and maximum growth rate predicted by the GRH [28]. However, this does not necessarily imply that fast-growing strains had a richer cell content in phosphorus, or that they

were more limited by phosphorus than were slow-growing bacteria. Lacking an increase in phosphorus content with μ_{max} in our evolved strains, their higher μ_{max} could not be attributed to the evolution of higher baseline abundances of P-costly structures such as ribosomes. The timescale of our experimental evolution might have been too short to produce heritable changes in phosphorus content. Moreover, our selection treatment for fast-growing bacteria evolved strong competitors for phosphorus, i.e. bacteria that produced more biomass per amount of phosphorus than did slow-growers. This is contrary to the weak competitors which would be expected under the GRH. Our result suggests that the potential for rapid growth is not linked to competitive ability for biosynthetic resources in such a straightforward manner as expected by the GRH [21].

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(b) Support for the size hypothesis

Transfers during the growing phase (low TV) did not simply select for high growth rates and associated resource allocation to biosynthesis function. Selection acted on a more integrative trait, cell size, which reversed the relationship between maximum growth rate and competitive abilities predicted by the GRH. This result strongly supports the SH, with a three-way trade-off among cell size, maximum growth rate and competitive ability for phosphorus (figure 1). Similar multiple-scale trade-offs involving size have been demonstrated by Edwards *et al.* [35] with empirical data on phytoplankton. This illustrates how hierarchies between different trade-offs may shape the relationships between life-strategy traits [19]. Fast-growing strains selected during the growing phase displayed smaller cell sizes than slow-growing strains. This is consistent with the hypothesis that smaller size may shorten the time needed for cell duplication [31,32]. In addition, if small size increases nutrient acquisition efficiency through a greater surface : volume ratio [29,34,35], it may feedback positively to biosynthesis. Interestingly, our ancestral strains' average cell size was larger than that of fast-grower evolved strains, suggesting that the largest cells grew too slowly to persist when the transfers occurred early during the growth period. This result shows that cell size is not only a plastic trait which would track variations in cell division rates through the growth period. Smaller bacteria should also be more abundant during the growing phase, because they grow faster in non-limiting conditions. Given the negative relationship between size and ability to grow fast, and size and nutrient acquisition mentioned above, it is not surprising that smaller 'fast-competitors' (both fast-growers and strong competitors for nutrients) emerged from selection during the growing phase [35,45].

(c) Selection of large cells

We expected that according to the supply–demand model [46], drops in nutrient concentration during the stationary phase would favour small cells with greater acquisition abilities and lower resource demand. Yet even if small cells were still abundant, we observed an increased proportion of large cells resulting in a higher mean cell size (figure 3c). This pattern may be explained by sharp fluctuations in resource availability: when the transfer occurred after bacteria had reached their maximal density, cells experienced periods of starvation. Pulses of nutrient supply are known to enable the persistence of large individuals which are more able to store nutrients [47]. Many bacteria have the capacity to store P-rich polyphosphates in dense granules [48,49]. Although we did not undertake the measurements to test this hypothesis, we can reasonably argue that the P-rich medium (electronic supplementary material, table S3) might have allowed the evolution of such large cells with high-storage capacities [50]. In temporally fluctuating environments, fast-growers can coexist with storage strategists [36,51,52]. For storage strategists, 'luxury consumption' in periods of resource abundance fuels growth and maintenance during periods of scarcity. Luxury consumption has also been proposed as an optimal competitive strategy leading to the starvation of non-storing competitors [53]. Several authors have suggested that the increase of phytoplankton cell size at the end of blooming events may be caused by an increase in grazing pressure by zooplankton [8,54]. Our results suggest as an alternative explanation that larger cells may flourish due to storage benefits

when nutrients are depleted at the end of a bloom. This interpretation is consistent with the findings of Litchman *et al.* [45], which demonstrated that small and large cells could coexist in intermediate-frequency nutrient pulses. Their marine diatom model successfully reproduces the bimodal size distribution observed in upwelling areas, which are subject to large nutrient pulses. Where nutrient supply is more continuous, such as in lakes, diatoms are smaller, grow faster and are more competitive for nutrients [45]. We argue that in our experiment, transfers with different volumes mimicked a gradient of pulsed nutrient supply amplitudes, which may have led to changes in cell size similar to what has been previously observed for diatoms. Under low TV, the relative abundance and low variation of nutrient availability may have generated a bottleneck for competitive strategies favouring smaller fast-competitors for phosphorus. By contrast, under high TV, the pulsed nutrient supply may have promoted the coexistence of both smaller fast-competitors and larger slow-growing bacteria, which we assume to be starvation-resistant due to their storage capacity.

(d) Traits in nitrogen-limiting conditions

Surprisingly, we found no relationship between traits in strains tested on low-N medium ($\mu_{max}^{(low-N)}$ and C_N), or between these traits and relative cell size. Based on the phytoplankton study of Edwards *et al.* [35], we were expecting to obtain a three-way trade-off between cell size and competitive abilities for nitrogen and phosphorus. In particular, we expected a negative correlation between C_N and cell size, consistent with that observed for phosphorus. Instead, we observed that some strains with relatively large cell sizes were both the fastest growers on low-N medium and were highly competitive for nitrogen, contradicting both the GRH and the SH. In the absence of additional data, we can only hypothesize that the pattern is a product of either evolution history or of interaction between focal traits and others.

Regarding evolution, our timescale may have been too short for selection to produce optimal adaptation. The environmental constraints imposed by the treatments may also not have sufficiently diverged regarding nitrogen use, for the optima to result in a trade-off line. In addition, the absence of control for the length of evolution among treatments (likely resulting in differing population sizes and generation numbers) probably led to among-strain variability in the final distance to these evolutionary optima. Only a strong trade-off would have been visible through such noise.

Regarding trait interaction, there may, for instance, be synergy between nitrogen and phosphorus with respect to nutrient acquisition. It has been shown for the bacterium *Pseudomonas aeruginosa* that polyphosphates stored in large cells may be mobilized for cell motility [55]. This might increase the capture of scarce nitrogen, which in turn would feedback positively on phosphorus acquisition [56,57]. In our case, this would have increased both μ_{max} and C_N of large cells relative to others, but only when phosphorus is abundant. Another possible mechanism would be that bacteria allocate more resources in growth machinery under nitrogen scarcity, because of a relatively higher nitrogen cost of uptake machinery [58]. This plastic response would maximize both nitrogen use efficiency and growth rate, invalidating the GRH's prediction of a trade-off. The last possible explanation is that nitrogen limitation may have a negative feedback on phosphorus uptake

[56,57] and thus biomass production regardless of strain characteristics. This would limit the relative advantage of small fast-growing cells over large cells for nutrient acquisition, and may have tempered the differences in competitive ability for nitrogen between our evolution treatments.

(e) Conclusion

Common approaches characterizing the link between maximum growth rate and competitive ability for nutrients include multi-taxa comparative analyses and experiments where diverse species are grown in contrasting conditions of nutrient availability. But evolutionary history introduces another relationship between traits, namely that traits may be similar in sister species for reasons unrelated to environmental adaptation. Here, the evolution of diverse competitive strategies from a single ancestral population enabled us to isolate the effect of physiological constraints on trait linkages. Our results strongly support the SH, and do not support the GRH, at least at the timescale considered. The evolution of competitive strategies was closely associated with the evolution of cell size in variable environments, which in turn tightly shaped a positive relationship between maximum growth rate and competitive ability for phosphorus. More generally, our study illustrates the potential for metabolic evolution of organisms in response to environmental constraints, and stresses the complex functional constraints underlying competitive strategies and species coexistence mechanisms.

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