

Increased survival during famine improves fitness of bacteria in a pulsed-resource environment

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ABSTRACT

Background: Organisms may experience alternating periods of feast and famine determined by variation in both resource supply and community composition. Environments with rare and large resource pulses may select for rapid growth during resource abundance and survival during resource scarcity. However, trade-offs may prevent individuals from investing in both traits equally.

Questions: Does the selective response of rapid-growth ability, or the ability to endure resource deprivation, dominate in an environment with rare resource pulses? Does the response depend on pulse amplitude? Does it also depend on whether a species faces only intra-specific competition or both intra- and inter-specific competition?

Study organisms: Two heterotrophic bacterial species – a gleaner (*Novosphingobium capsulatum*) and an opportunist (*Serratia marcescens*).

Methods: We imposed 7-day resource renewal cycles with either high- or low-amplitude fluctuations in resource availability. We cultured the bacteria in one-species monocultures or in two-species communities. We measured the fitness of ancestor strains and evolved strains in 7-day assays that mimicked the environments of the selection experiment.

Results: Both species rapidly evolved a prudent strategy: descendants had both larger populations and better survival than ancestors. In addition, when growing with *S. marcescens*, *N. capsulatum* had an increased growth rate in environments with larger resource fluctuations. Otherwise, the maximum growth rate of neither species responded to the experiments.

Conclusion: Survival during low-resource conditions can be a key community context-dependent trait in fluctuating environments. We found no trade-off between growth rate during feast and survival during famine.

Keywords: competition, *Novosphingobium capsulatum*, population dynamics, *Serratia marcescens*, trade-offs.

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INTRODUCTION

Resource pulses are temporal events of increased resource availability with low frequency, short duration, and large magnitude (defined by Yang *et al.*, 2008). Resource pulses have been described in different types of natural habitats – in terrestrial ecosystems from arid deserts to tropical forests, and in aquatic environments from rivers and lakes to marine ecosystems (reviewed by Yang *et al.*, 2008). Although resource pulses are relatively common in nature, their effects on population and community dynamics, especially on an evolutionary time scale, are not thoroughly understood (Yang *et al.*, 2008). Resource pulses may lead to alternating periods of resource abundance and scarcity. Variation in resource availability is predicted to fundamentally affect competitive interactions and community composition (Levins, 1979; Chesson and Huntly, 1997). The alternating ‘feast’ and ‘famine’ periods potentially select for different growth strategies and may enable the long-term co-existence of species (Roughgarden, 1971; Chesson and Huntly, 1997).

The effects of resource fluctuations on individual, population, and community levels depend on the frequency and magnitude of fluctuations (Levins, 1979; Chesson and Huntly, 1997; Holt, 2008). Resource pulses may temporarily enable rapid growth and large population size but may also increase extinction risk in closed systems where dispersal is limited (Holt, 2008). First, immediately after the resource pulse, population size increases. Then, if the pulse interval is long, a large population may over-exploit the resources. This may lead to more intense resource competition, and if the population is not able to survive through the trough, even extinction is plausible (Holt, 2008; Yang *et al.*, 2008). If the resource pulses are rare and have large amplitude, organisms may benefit from the resource abundance immediately after the pulse, but they also have to cope with the following resource scarcity during the trough (Yang *et al.*, 2008). The magnitude and the frequency (in relation to generation time) of resource fluctuations are predicted to affect the evolutionary changes in life-history traits (Kassen, 2002). On an evolutionary time scale, the different phases of the resource pulse may cause a selective pressure on different traits. Shortly after the pulse, a quick response to the increased amount of resource and rapid growth are beneficial, and later, during the trough of the pulse, the ability to withstand low resource conditions and efficient use of available resources enable survival (Yang *et al.*, 2008). Furthermore, trade-offs may prevent individuals from allocating resources to several of these traits simultaneously (Stearns, 1989; Roff and Fairbairn, 2007).

We investigated how the resource pulses and competitive environment affect long-term population dynamics, and the evolution of life-history traits in bacterial populations. Bacteria have relatively short generation times and large populations can be cultivated in laboratory conditions. For these reasons, they are the ideal model organisms for studies of long-term population dynamics (e.g. Spencer *et al.*, 2005; Carrero-Colón *et al.*, 2006; Hiltunen *et al.*, 2008; Trosvik *et al.*, 2008) and experimental evolution (e.g. Gause, 1934; Luckinbill, 1978; Lenski *et al.*, 1991; Vasi *et al.*, 1994; Novak *et al.*, 2006; Friman *et al.*, 2008). Furthermore, as bacteria can be stored indefinitely in suspended animation, it is possible to form living libraries of bacterial strains from different instants during their evolutionary history (Lenski and Travisano, 1994).

In this study, we exposed two bacterial species, *Novosphingobium capsulatum* and *Serratia marcescens*, to periodic resource fluctuations for 13 weeks, representing approximately 2000 generations. Species were grown as one-species monocultures and two-species communities in batch cultures. The resource environment was renewed weekly, which caused a pulse-like resource inflow to the system. We measured the population-level fitness estimates for the

ancestors and the evolved strains of both species in separate 7-day assays. We used the time lag before the population reached its maximum growth rate and the maximum growth rate during resource abundance as measures for an immediate response to the resource pulse. Survival after the population had reached its maximum size and the population size at the end of the 7-day measurement were used as proxies for resistance to harsh resource conditions.

In a regularly fluctuating, pulsed environment, the amplitude of the resource pulse affects the length of the period when resources are scarce; if the pulses are small, the periods of resource shortage are longer than if the pulses are large. Our main hypothesis is that when the resource pulse amplitude is small and the period of low resources is relatively long, there is selection for an economical resource utilization strategy and increased survival. In contrast, when the resource pulses are large, the ability to grow quickly during the period of resource abundance could provide a competitive edge. The intensity of within- and between-species competition may further shape the evolutionary outcome. We address three questions: (1) How does the magnitude of a resource pulse shape the long-term population dynamics and evolutionary changes in life-history traits? (2) What are the direction and the magnitude of change in growth during resource abundance and in survival during resource deprivation? (3) Does the community composition alter the long-term dynamics?

The species in our study differ in their growth strategies – *N. capsulatum* is a gleaner, whereas *S. marcescens* is an opportunist. Based on these differences in the functional response to resource availability, *S. marcescens* should be competitively superior during resource abundance (shortly after the resource pulse) and *N. capsulatum* during resource scarcity (the late phase of the pulse). *Serratia marcescens* is expected to grow rapidly during resource abundance, which could lead to high population densities, especially in the large resource pulse environments. Thus, the density-dependent competition during resource abundance is potentially more intense in the two-species systems and in the *S. marcescens* monocultures. *Novosphingobium capsulatum* is expected to have better survival during resource scarcity. If *S. marcescens* does not outcompete *N. capsulatum* during resource abundance in the two-species communities, the competitive ranking between species is likely to change during resource scarcity in favour of *N. capsulatum*.

METHODS

Long-term experiment

Two bacterial species, *Serratia marcescens* (from American Type Culture Collection strain ATCC 13880) and *Novosphingobium capsulatum* (ATCC 14666), were grown as monocultures and in a two-species community in aquatic batch cultures on a detritus resource in a 7-day periodic environment. These bacteria are heterotrophic, Gram-negative, rod-shaped, and do not form spores. *Serratia marcescens* is a facultative anaerobe and belongs to the family Enterobacteriaceae (Grimont and Grimont, 1978; Krieg and Holt, 1984). The ATCC strain of *S. marcescens* was originally isolated from pond water. *Novosphingobium capsulatum* is aerobic and belongs to the family Sphingomonadaceae (Leifson, 1962; Takeuchi *et al.*, 2001). The *N. capsulatum* strain was originally isolated from distilled water (Leifson, 1962). These species can be separated based on colony morphology: *S. marcescens* forms white, pink or red colonies, whereas *N. capsulatum* forms yellow colonies when grown on nutrient broth agar plates. They have different growth responses to a fresh cereal leaf medium: *N. capsulatum*

grows faster than *S. marcescens* at a low concentration, and *S. marcescens* grows faster at intermediate and high concentrations [Monod parameters estimated from the measured growth rates in 0.1–1.0 g·L⁻¹ hay extract are maximum growth rate, $r_{\max} = 0.103 \pm 0.047$ and half-saturation constant, $K_s = 0.29 \pm 0.36$ for *N. capsulatum* and $r_{\max} = 0.418 \pm 0.157$ and $K_s = 1.72 \pm 0.89$ for *S. marcescens*; mean $\pm$ S.E.M. (Hiltunen *et al.*, 2008)].

Bacteria were grown in an environment where the weekly resource renewal caused a pulse-like resource inflow. Two resource pulse amplitudes were used: 99.9% or 70% of the total volume of microcosms was replaced with a fresh growth medium. The experiment was continued for 13 renewals, representing approximately 2000 bacterial generations based on an estimated average growth rate of one generation in 1 h. In the large-amplitude resource pulse treatment, 150 μ L of the old medium was weekly transferred to 150 ml of a fresh medium, including a bacterial population of approximately $2 \times 10^5 \pm 2 \times 10^4$ colony forming units (CFU)·ml⁻¹ in the *S. marcescens* only treatment, $6 \times 10^4 \pm 8 \times 10^3$ CFU·ml⁻¹ in the *N. capsulatum* only treatment, and $1 \times 10^5 \pm 3 \times 10^4$ CFU·ml⁻¹ in the two-species treatment. Correspondingly, in the small-amplitude resource pulse treatment, 45 ml of the old medium was transferred to 105 ml of a fresh medium, including a population of approximately $6 \times 10^7 \pm 6 \times 10^6$ CFU·ml⁻¹ in the *S. marcescens* only treatment, $3 \times 10^7 \pm 4 \times 10^6$ CFU·ml⁻¹ in the *N. capsulatum* only treatment, and $4 \times 10^7 \pm 4 \times 10^6$ CFU·ml⁻¹ in the two-species treatment. In all treatments, the initial population sizes were relatively large, and thus the amount of demographic stochasticity was likely to be negligible. All treatments had three replicates.

The microcosms were filter-capped, 250-ml cell culture bottles (Corning) containing 150 ml of phosphate-buffered cereal leaf extract. The medium was prepared as follows: 1 g·L⁻¹ of cereal leaf powder (Ward's Natural Science, Rochester, NY) was boiled for 10 min in de-ionized H₂O, cooled, and filtered through a glass microfibre filter (GF/C, Whatman). The filtering procedure leaves a 2.15 mg·L⁻¹ dry weight of cereal leaf powder in the final medium. Phosphate buffer adjusted to pH 7.5 (1.57 g·L⁻¹ of K₂HPO₄·3 H₂O, 0.4 g·L⁻¹ of KH₂PO₄, 0.5 g·L⁻¹ of (NH₄)₂SO₄, 0.1 g·L⁻¹ of MgSO₄·7 H₂O, 0.01 g·L⁻¹ of NaCl, and 0.023 g·L⁻¹ of CaCl₂·2 H₂O in de-ionized H₂O) was added to the medium. The medium was autoclaved at 121°C for 20 min. Prior to the inoculation in the growth medium, the bacteria were incubated for 3 days on agar plates (10 g·L⁻¹ of nutrient broth [Difco™, BD], 2.5 g·L⁻¹ of yeast extract, and 15 g·L⁻¹ of agar [Scharlau Chemie S.A.] in de-ionized H₂O). Approximately 50 colonies were streaked from an agar plate and mixed in sterile, phosphate-buffered, de-ionized H₂O. The starting population sizes were 0.1% or 30% of the estimated maximum population size, corresponding to the pulse amplitudes. For the two-species community, the species were mixed in a 1:1 biomass ratio. The microcosms were kept at 25°C. The relatively low concentration of the detritus resource and the volume-to-surface area ratio of the microcosms suggest that oxygen was available throughout the experiment in all parts of the microcosms. During the weekly resource renewals, a 600 μ L sample of the growth medium from each experimental unit was aseptically mixed in sterile freezing solution. The sample contained living cells and was stored in suspended animation at -70°C. The freezing solution contained nutrient medium (10 g·L⁻¹ of nutrient broth [Difco™, BD], 1.25 g·L⁻¹ of yeast extract [Scharlau Chemie S.A.] in de-ionized H₂O), and glycerol (99% w/v; VWR) in a volume ratio of 1:5.

Fitness assays

We measured the population growth of the ancestors and the evolved bacterial strains with 1-week and 13-weeks histories in the long-term experiment in separate week-long fitness assays. The growth medium in the assays was phosphate-buffered hay extract medium, identical to the medium in the long-term experiment.

We inoculated ancestral bacteria for the fitness assay as described in the long-term experiment. For the evolved bacterial strains, we used a modified protocol that allowed us to reliably separate the two bacterial species from the two-species communities. The bacteria from the long-term experiment were thawed and incubated on agar plates ($10 \text{ g} \cdot \text{L}^{-1}$ of nutrient broth in de-ionized H_2O , details as above) for 2 days at 25°C . From these plates, approximately 30 randomly selected colonies ($10 \mu\text{L}$ of bacterial biomass) were mixed in phosphate-buffered de-ionized H_2O , serially diluted, plated, and then incubated for 3 days at 25°C on agar plates. Thereafter, the inoculation was done similarly as at the start of the long-term experiment. During the fitness assays, the samples of living cells were taken at 0, 5, 10, 20, 30, 40, 50, 70, 100, and 168 h after inoculation of the bacteria. At each sampling, we aseptically transferred 0.5 ml of the medium, mixed it with 0.5 ml of sterile freezing solution, and stored it in suspended animation at -70°C . We measured the population sizes from these samples based on a standard serial dilution-plating procedure. The dilution solution was phosphate-buffered, de-ionized H_2O . The bacteria were incubated for 3 days on agar plates ($1 \text{ g} \cdot \text{L}^{-1}$ nutrient broth) at 25°C before counting the colony forming units. The colony forming units were calculated as a weighted mean of all platings per sample, and the plating dilution coefficient was used as the weight because in a serial dilution plating, the variability of the CFU estimate increases with an increasing dilution coefficient.

We calculated four growth performance variables from the population-size data (shown in Fig. 1): the maximum growth rate at the beginning of the growth cycle, the time-lag before the maximum growth rate was achieved, the population size at the end, and the mortality after the maximum population size was reached. To calculate the maximum growth rate, a linear regression line was fitted to the logarithm of the population size data of each replicate population during the 0–50 h of growth. The time lag equalled a period from the start (0 h) to the mid-point of the time-window used in the fitting of the regression line. The mortality was calculated as the difference between the maximum population size and the population size at the end of the cycle divided by the maximum population size.

Statistical analyses

We modelled the effects of the time spent in the long-term experiment, the amplitude of the resource pulse, and the species-composition treatments on the measured variables (mortality, end population size, length of the lag phase, and maximum growth rate) using a Linear Mixed Model procedure in SPSS v.16 (SPSS Inc., Chicago, IL). We constructed the models separately for each species. The models included a repeated factor and fixed factors with all two- and three-way interactions. The repeated factor was the week from which bacterial strains originated (ancestor strains, strains after 1 week, or 13 weeks in the long-term experiment) and the experimental units were the repeated subjects. Time and the two treatments were set as the fixed effects. The end population size was log transformed and the

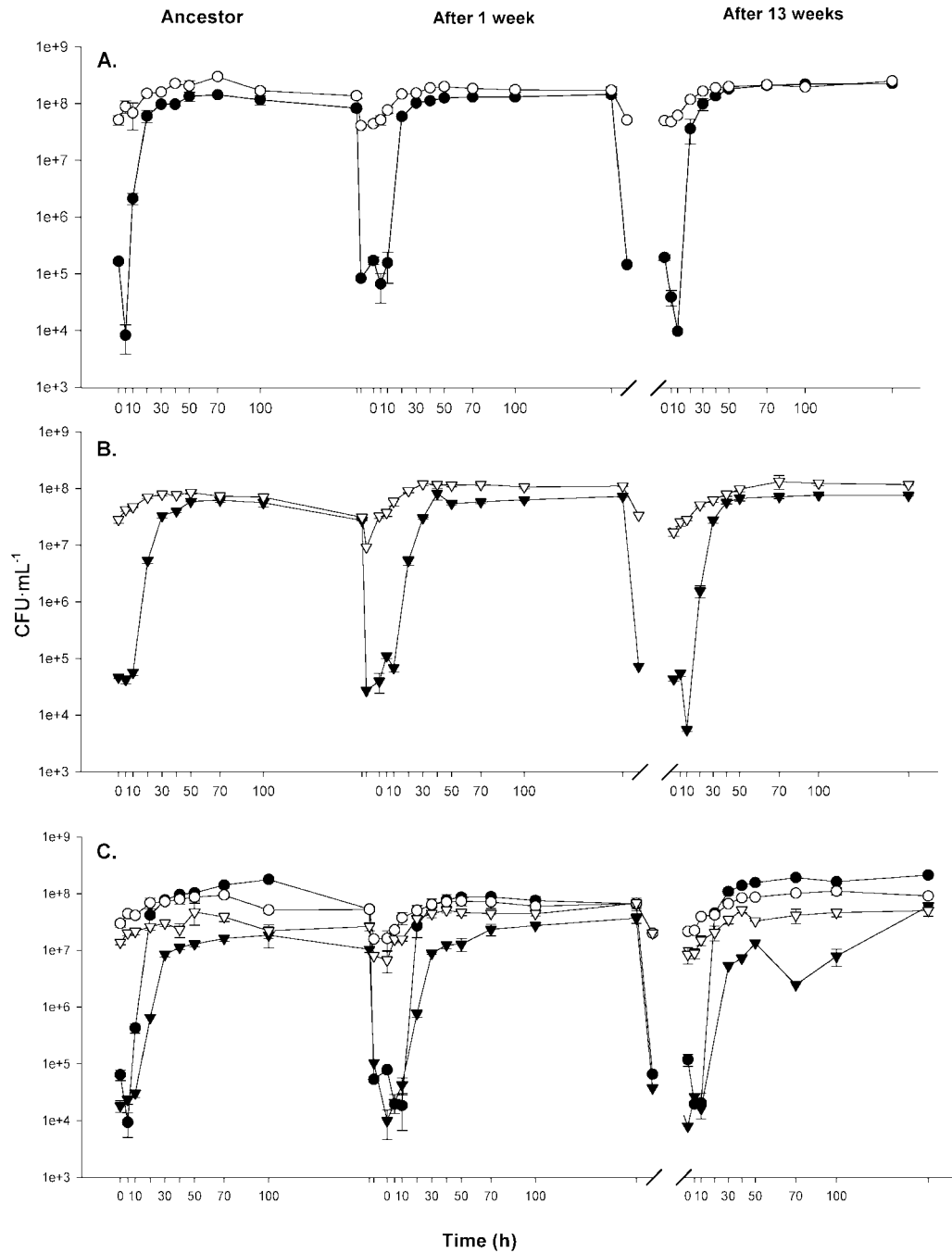


Fig. 1. Population sizes as colony forming units (CFU·mL⁻¹ ± 1 S.E.M.) for ancestor and evolved strains of (A) *Serratia marcescens* growing alone, (B) *Novosphingobium capsulatum* growing alone, and (C) the two species growing together during 7-day fitness assays. The population sizes of the ancestor strains are on the left and of the evolved strains after 1 week or 13 weeks in the long-term experiment are in the middle and on the right, respectively. ●, *S. marcescens*, large pulse; ○, *S. marcescens*, small pulse; ▼, *N. capsulatum*, large pulse; △, *N. capsulatum*, small pulse.

mortality arcsine transformed in the analyses. A compound symmetry was used as the model covariance structure in all models except for the time lag and mortality, for which an unstructured covariance was used.

RESULTS

Mortality

The evolved strains of both species had a lower mortality than their ancestors in all treatments, and the strains that evolved 1 week or 13 weeks in the pulsed-resource environment did not differ from each other (time main effect: *S. marcescens*, $F_{2,8.43} = 109.74$, $P < 0.001$, Fig. 2A; *N. capsulatum*, $F_{2,8.24} = 17.85$, $P = 0.001$, Fig. 2B). The mortality of all *S. marcescens* strains was lower when grown in the monocultures than with *N. capsulatum* (mortality: $14 \pm 2\%$ and $23 \pm 2\%$ of the maximum population size $\pm$ s.e.m., respectively; community effect: $F_{1,8.6} = 8.93$, $P = 0.016$). The mortality of *S. marcescens* changed over time and depended on the pulse amplitude: the ancestor had the highest mortality in the two-species communities with large resource pulses, whereas after 13 weeks the highest mortality was observed in the strains from the two-species communities that had experienced small resource pulses (Fig. 2A). Furthermore, when the resource pulse amplitude was large, the 1-week strains from the two-species communities had a higher mortality than the strains from the monocultures, but when the resource pulse amplitude was small, community composition did not have an effect on mortality (time $\times$ pulse $\times$ community effect: $F_{2,8.43} = 7.07$, $P = 0.016$, Fig. 2A). Resource pulse amplitude or community composition alone did not affect *N. capsulatum* mortality.

End population size

The end population sizes after 1 week of culturing were largest in the monocultures (community main effect: *S. marcescens*, $F_{1,8} = 102.40$, $P < 0.001$, difference of $6 \times 10^7 \pm 6 \times 10^6$ CFU $\cdot$ mL⁻¹ $\pm$ s.e.m.; *N. capsulatum*, $F_{1,4.75} = 92.16$, $P < 0.001$, difference of $2 \times 10^7 \pm 2 \times 10^6$ CFU $\cdot$ mL⁻¹). The *N. capsulatum* strains from the small resource pulse environments reached a higher end population size than the strains from the environments with large pulses ($6 \times 10^7 \pm 2 \times 10^6$ CFU $\cdot$ mL⁻¹ and $4 \times 10^7 \pm 2 \times 10^6$ CFU $\cdot$ mL⁻¹, respectively; pulse effect: $F_{1,4.75} = 39.68$, $P = 0.002$).

The evolved strains of both species had larger end population sizes than their ancestors (time main effect: *S. marcescens*, $F_{2,16} = 59.29$, $P < 0.001$, Fig. 3A; *N. capsulatum*, $F_{2,12.44} = 87.81$, $P < 0.001$, Fig. 3B). The effect of community composition on the end population size of *S. marcescens* changed so that the end population size of the 13-week evolved strains from the two-species communities and the large resource pulse environment did not differ from the monocultures (community $\times$ time effect: $F_{2,16} = 2.33$, $P = 0.129$, Fig. 3A). The evolutionary increase in the end population size of *S. marcescens* was larger if the resource pulses were large compared with the environment with small resource pulses (time $\times$ pulse effect: $F_{2,16} = 7.44$, $P = 0.005$, Fig. 3A).

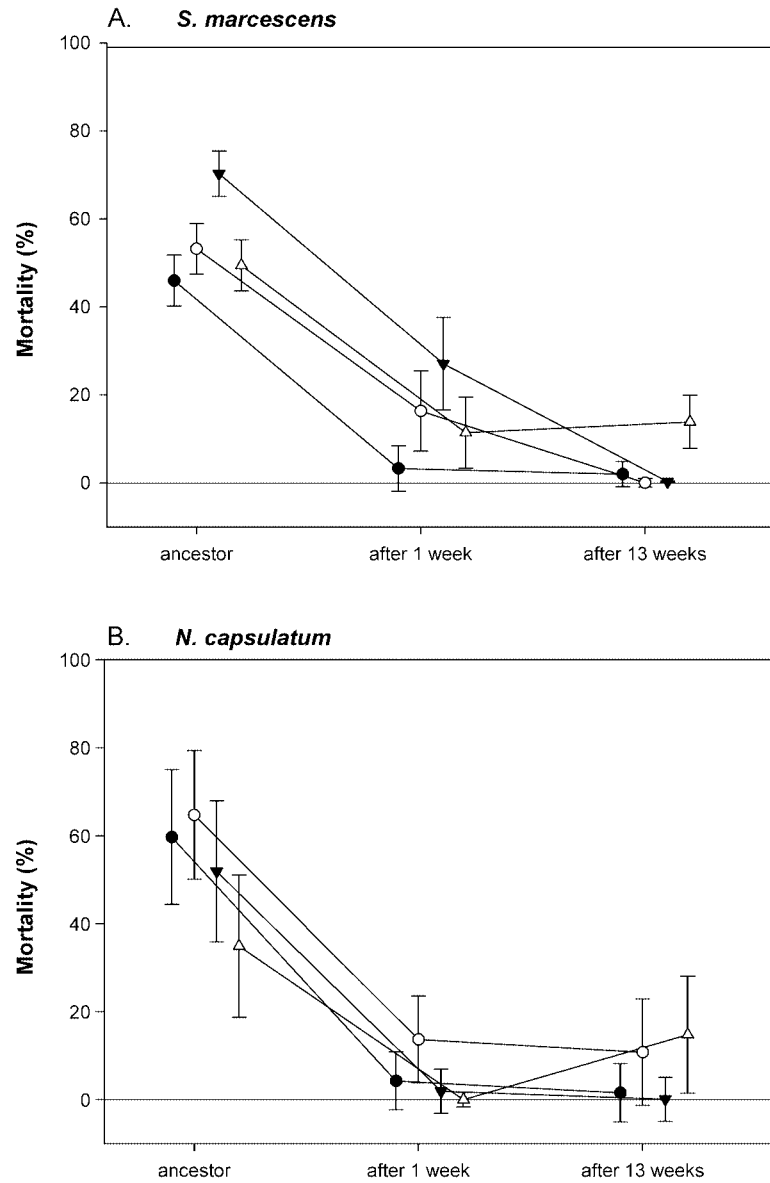


Fig. 2. Estimated marginal means for mortality after maximum population size during the 7-day growth period for (A) *S. marcescens* and (B) *N. capsulatum*. Marginal means are based on GLMM. On the x-axis is the identity of the bacterial strains: ancestor strain and strains after 1 week or 13 weeks of evolutionary history in the resource pulse environment. On the y-axis is mortality, which is calculated as the percent difference between maximum population size and end population size. Treatments: *large* and *small pulse* refer to the amplitude of the resource pulse; *one species*: strains have lived alone in monocultures; *two species*: strains have lived in a two-species community. Error bars for colony forming units indicate ± 1 s.e.m. ●, one species, large pulse; ○, one species, small pulse; ▼, two species, large pulse; △, two species, small pulse.

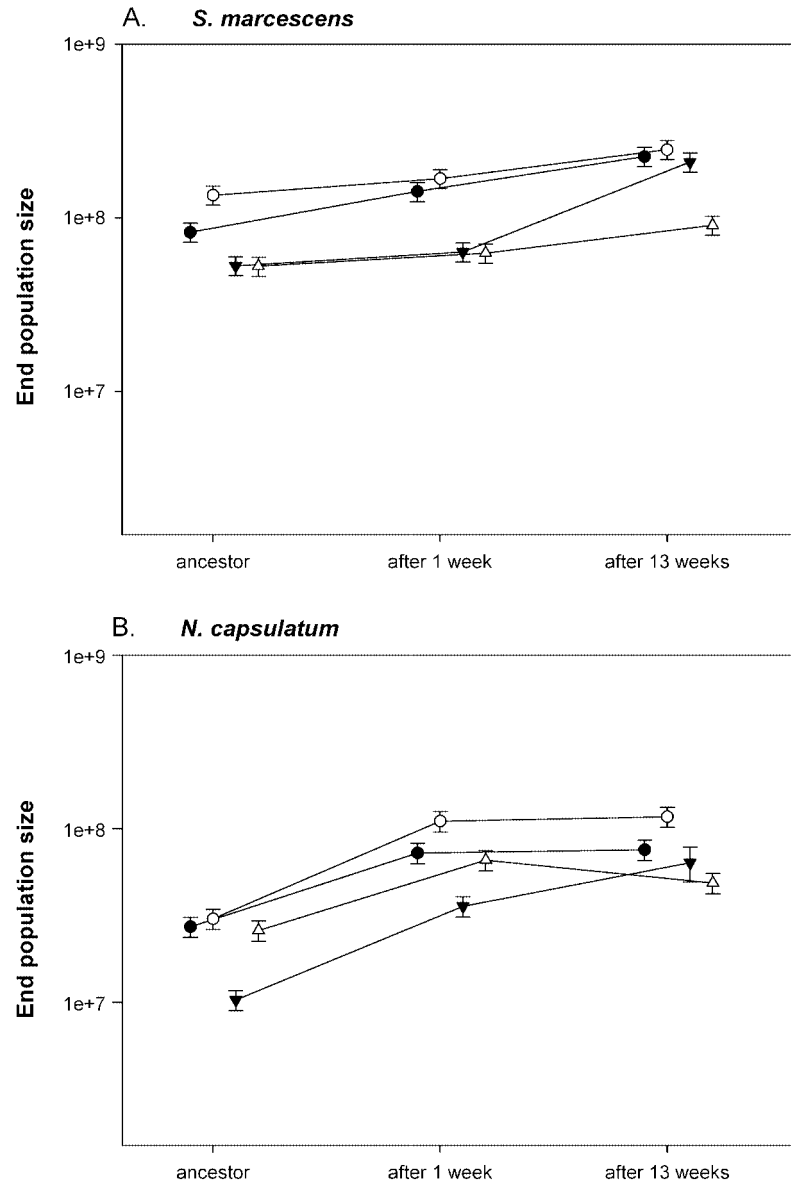


Fig. 3. Estimated marginal means for the end population size of (A) *S. marcescens* and (B) *N. capsulatum* in different treatments for ancestor and evolved strains. Marginal means are based on GLMM. On the y-axis is the end population size on a logarithmic scale. The treatments and x-axis are the same as in Fig. 2. Error bars indicate ± 1 S.E.M. ●, one species, large pulse; ○, one species, small pulse; ▼, two species, large pulse; △, two species, small pulse.

Time lag before the onset of maximum growth rate phase

The time lag of *S. marcescens* was shortest in the large resource pulse environments regardless of community composition (Fig. 4A). The time lag of *S. marcescens* was shorter in the small amplitude resource pulse environment (time $\times$ pulse effect: $F_{2,8} = 20.32$, $P = 0.001$, Fig. 4A). The community composition effects on the time lag of *N. capsulatum* varied temporally in the small amplitude resource pulse environments: for the ancestor, the time lag was longest in the two-species communities and the shortest in the monocultures. After 1 week, the order was reversed, and after 13 weeks, the shortest time lag was again seen in the monocultures (time $\times$ pulse $\times$ community effect: $F_{2,8.5} = 24.40$, $P < 0.001$, Fig. 4B).

Maximum growth rate

The maximum growth rates of the evolved strains and their ancestors did not differ from each other. The maximum growth rates of both species were highest in the large resource pulse environments (pulse main effect: *S. marcescens*, $F_{1,8} = 245.41$, $P < 0.001$; *N. capsulatum*, $F_{1,8.05} = 1434.94$, $P < 0.001$, Fig. 5). *Novosphingobium capsulatum* generally grew slightly faster in the monocultures than together with *S. marcescens* (0.07 ± 0.02 and 0.06 ± 0.02 h⁻¹ $\pm$ S.E.M., respectively; community effect: $F_{1,8.05} = 11.83$, $P = 0.009$). However, as an exception, the *N. capsulatum* strains from the 1-week condition had slightly higher growth rates if they grew in the two-species communities than in the monocultures (time $\times$ community effect: $F_{2,14.69} = 18.25$, $P < 0.001$, Fig. 5B).

DISCUSSION

In an environment where new resources become available rarely and organisms experience relatively long periods of resource shortage, the ability to endure low resource conditions can be under strong selection pressure. However, if the resources become available in large pulses, a quick response to the enrichment of resources and rapid growth on the high resources could be selected for. We tested how between-generation resource pulses affect the evolution of fitness-related traits such as growth rate and survival by exposing two heterotrophic bacterial species with contrasting growth strategies to repeated 7-day cycles of ‘feast’ and ‘famine’ for 13 weeks. Furthermore, the bacteria were grown in monocultures and two-species communities to test how the competitive environment affects the outcome of evolution in a variable environment. In the two-species communities, these species co-existed throughout the experiment, and here we discuss the possible mechanisms that enabled their long-term co-existence. The growth dynamics of the ancestor strains and the evolved strains after 1 week or 13 weeks in the long-term experiment were evaluated in separate 7-day fitness assays that mimicked the environmental conditions of the long-term selection environment. In the fitness assays, the evolved bacteria grew to higher end population sizes than their ancestors and had higher survival during the resource-deprivation phase. There was no difference in the maximum growth rates during resource abundance between the ancestors and the evolved strains. The marked improvements in survival during resource scarcity combined with the minor changes in growth rate during resource abundance suggest that survival is an important fitness component in environments with regular resource pulses. Interestingly, the main response, the evolutionary increase in

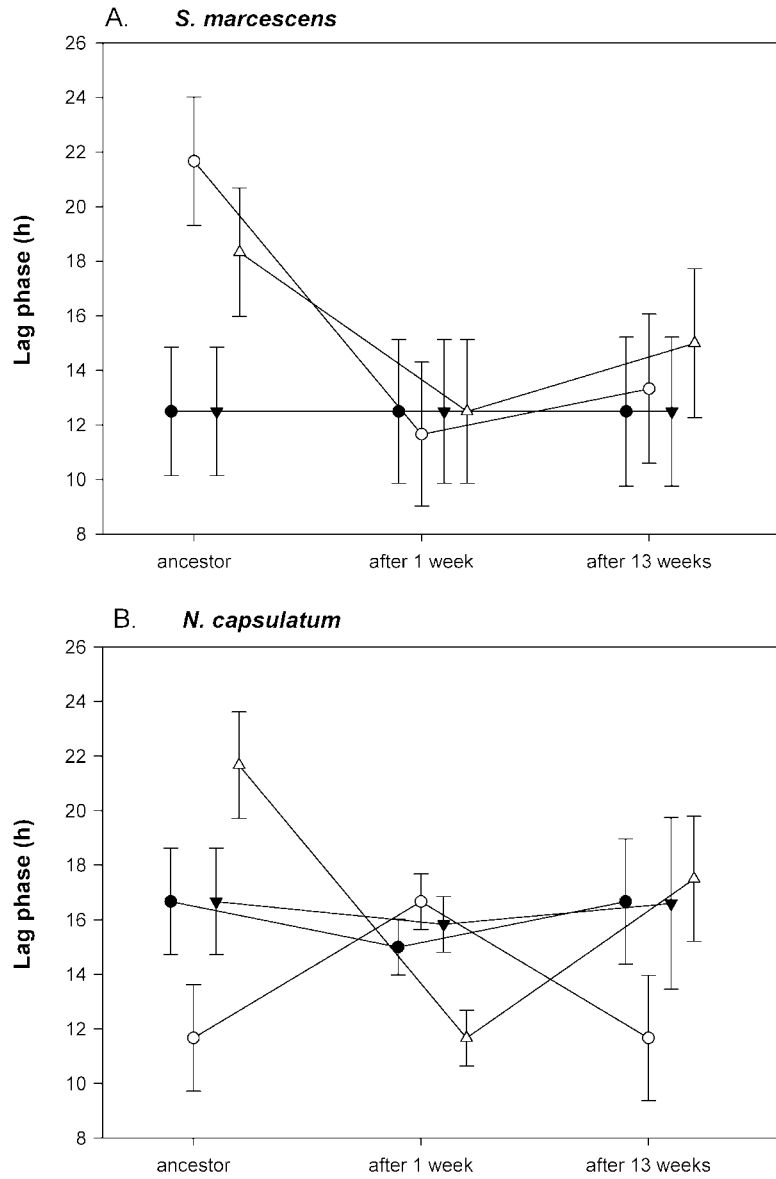


Fig. 4. Estimated marginal means for length of lag phase before reaching the maximum growth rate for (A) *S. marcescens* and (B) *N. capsulatum*. The treatments and x-axis are the same as in Fig. 2. Error bars indicate ± 1 S.E.M. ●, one species, large pulse; ○, one species, small pulse; ▲, two species, large pulse; △, two species, small pulse.

survival during resource scarcity, was similar irrespective of the intrinsic differences in the growth strategies of the studied species, the amplitude of the resource pulse, or the community composition. In general, the survival of *S. marcescens* was higher in the monocultures than when grown with *N. capsulatum*, but during the long-term experiment, survival increased in both treatments. In the monocultures, the end population sizes of both

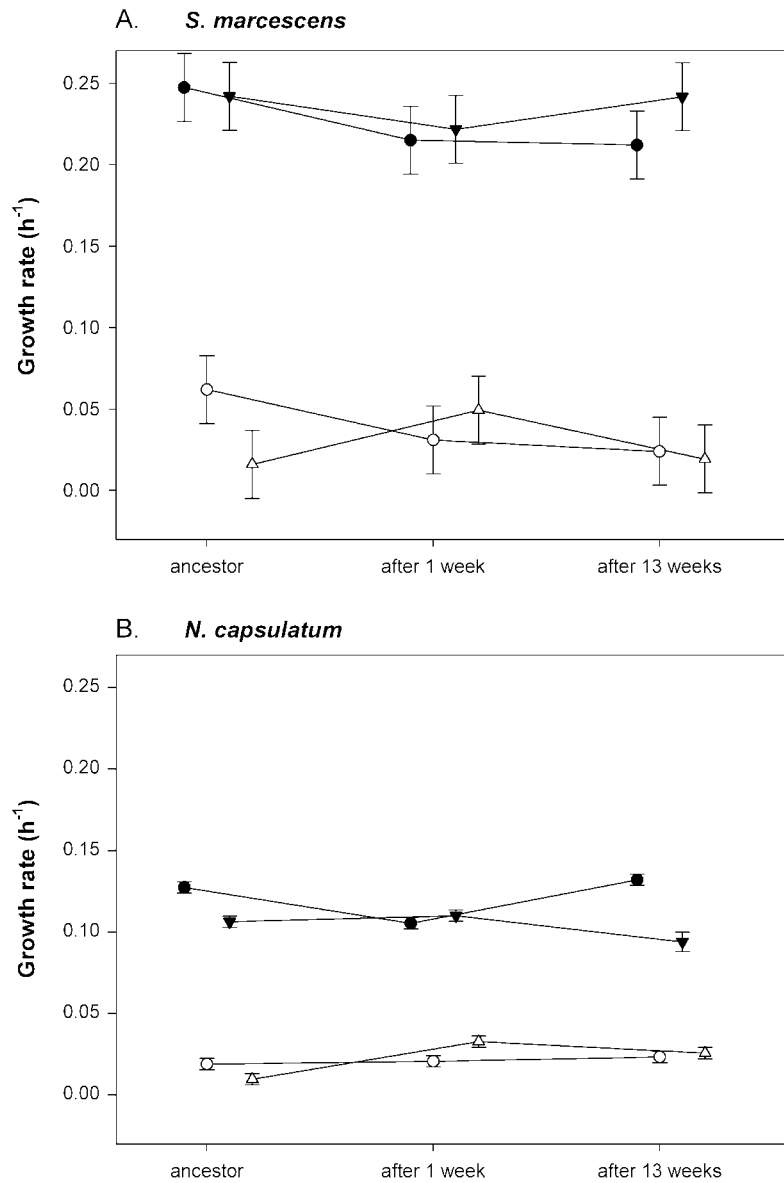


Fig. 5. Estimated marginal means for maximum growth rate for (A) *S. marcescens* and (B) *N. capsulatum*. The treatments and x-axis are the same as in Fig. 2. Error bars indicate ± 1 S.E.M. ●, one species, large pulse; ○, one species, small pulse; ▲, two species, large pulse; △, two species, small pulse.

species were larger than when grown together, which could result from inter-specific resource competition between these species. Furthermore, as we found no evidence for impaired growth performance between the evolved strains when resources were abundant, it is possible that in this system, there is no apparent trade-off between growth performance in resource-rich environments and survival during resource deprivation.

The evolutionary increase in survival has been reported also in other long-term studies where bacteria have been exposed to temporal resource fluctuations (Vasi *et al.*, 1994; Finkel, 2006; Novak *et al.*, 2006; Rozen *et al.*, 2009). One motivation in these studies and the present one has been an attempt to gain information on the long-term dynamics in microbial communities to better understand how evolutionary changes in life-history traits affect diversity and community dynamics. Our results highlight the significance of survival during the resource deprivation phase in environments where new resources become available rarely. These results are in concordance with the two-phase resource hypothesis of plant interactions along productivity gradients (Goldberg and Novoplansky, 1997). This hypothesis describes how the pulse and the inter-pulse periods affect growth dynamics and competitive interactions in plant communities and identifies survival during the inter-pulse as a critical biological challenge for organisms that experience periods of resource shortage (Goldberg and Novoplansky, 1997). For example, in arid environments with periodic rainfall, plants experience pulsed dynamics in resource availability, which is prone to influence community dynamics and diversity (Chesson *et al.*, 2004). Thus, there should be a selection pressure for mechanisms that increase survival during the resource shortage. It is not straightforward, however, that enhanced survival in low resource conditions is always due to selection pressure acting on that specific trait. For example, Vasi *et al.* (1994) reported the evolution of enhanced survival in resource-poor environments in *Escherichia coli* strains, but stated that ‘this trait cannot be considered a fitness component *per se*, since it is manifested only in environments different from the environment in which populations evolved’. Vasi *et al.* (1994) used an experimental system where the resource renewal cycle was only 24 h, and hypothesized that the selection pressure was for enhanced growth rate, and not so much survival. Thus, as enhanced survival during low resource conditions seems to evolve also in environments where prolonged resource deprivation is unlikely, it is possible that increased survival is linked to some other beneficial trait. In our experiment, genotypes that were not able to survive during the low resource conditions were lost between the resource renewals, and thus, there should be strong selection pressure on properties that enhance survival. Furthermore, as we did the fitness assays in an environment similar to the selection environment, the performance should reflect the actual fitness in the environmental conditions during the long-term experiment. Thus, our findings on enhanced survival during resource scarcity most likely resulted from selection on this particular trait.

It is well documented that certain fitness-related traits such as growth rate during resource abundance evolve in environments where resource availability fluctuates over time (Luckinbill, 1978; Lenski *et al.*, 1991; Vasi *et al.*, 1994). There is also a sound theoretical basis predicting the existence of trade-offs between such fitness-related traits that are energetically costly to produce and maintain (see Roff and Fairbairn, 2006 and references therein). However, empirical studies on the evolution of trade-offs are scarce (but see Buckling *et al.*, 2006; Novak *et al.*, 2006; Friman *et al.*, 2008). Previous studies using *E. coli* have shown that both the ability to grow rapidly immediately after nutrient enrichment (Vasi *et al.*, 1994) and mechanisms that enhance survival during resource deprivation can evolve in a periodically fluctuating environment (Finkel, 2006; Rozen *et al.*, 2009). We believe it noteworthy that none of the traits we measured in this experiment showed impairment, indicating that the measured traits are not traded off against each other. This is in contrast with other studies on evolutionary changes in growth dynamics of bacteria that have reported negative correlations between some of the measured fitness traits, indicating the existence of trade-offs (Vasi and Lenski, 1999; Buckling *et al.*, 2006; Novak *et al.*, 2006; Friman *et al.*, 2008). Trade-offs might be more easily detectable when bacteria are grown in

contrasting environments. For example, the starvation-selected mutant strains of *E. coli* were inferior competitors than their ancestors when grown in a rich-resource environment, but competitively superior when grown on low resources (Vasi and Lenski, 1999). Vasi and Lenski (1999) suggested that these environment-dependent differences in the competitive ranking of the evolved strains and their ancestors could be due to a common trade-off between adaptation to prolonged starvation and the competitive ability in high resource conditions. Our results contradict their findings, as we did not detect trade-offs between survival and growth rate. Here, fitness assays lasted for a week, mimicking the feast and famine cycle in the long-term experiment, and the resource environments in both the long-term experiment and fitness assays were identical. In batch cultures, bacteria experienced both resource abundance and scarcity during the week's growth. Both *S. marcescens* and *N. capsulatum* had their maximum growth rates during approximately the first 24 h after inoculation, and there was no consistent difference in the time lag before reaching the maximum growth rate between the ancestors and the evolved strains. If bacteria have experienced both high and low resource conditions during their evolutionary history, and their fitness is tested in an environment similar to the selection environment, the possible trade-off in survival during starvation and growth rate during high resource conditions should become clear. It is conceivable that trade-offs between survival and growth rate existed, but we failed to capture them as we did not test the growth performance in novel environments. Also, the time that selection in certain environmental conditions is allowed to continue might make the trade-off between traits more profound due to the accumulation of deleterious mutations (Reboud and Bell, 1996). Interestingly, here, most differences between the ancestors and the evolved strains were notable already after the 1-week growth cycle, approximately 150 generations, and thereafter only minor changes in growth performance were observed. This may indicate that the selection rapidly eradicated existing variation in populations.

Both species responded to the resource fluctuations by increasing their survival during low resource conditions. In general, microbes often respond to resource scarcity by forming non-growing resistant forms such as spores (McArthur, 2006) and reducing their metabolic activity (Vasi and Lenski, 1999; Ferenci, 2001) or by scavenging and cannibalism (Rozen *et al.*, 2009). The species in our study do not form spores and thus the enhanced survival could be due to changes in metabolic activity or an enhanced capability to use the complex resource medium (McArthur, 2006). However, whether changes in the population dynamics after the population reached its maximum size were due to better survival (cells survived longer but did not reproduce) or changes in the cell turnover rate cannot be shown from the data. Furthermore, the magnitude of change in any fitness character depends on both the selection pressure and the existing potential for change in that trait. Heritable changes in population end size after the 1-week growth as well as in survival ability were quick and profound, which could indicate strong selection pressure acting on those traits. As these changes took place over ≈ 150 generations, it is likely that the potential for change in these traits existed in the ancestor strains on a genetic level and was not caused by the accumulation of novel mutations. In contrast, there was no consistent difference in the maximum growth rates between the evolved and the ancestor strains of either species, *S. marcescens* or *N. capsulatum*. One plausible explanation is that the ancestor strains of these bacteria were already close to their theoretical maximal growth rates, maybe due to the history of laboratory culturing in high-resource conditions, and thus there was little room for improvement in their growth ability.

How environmental fluctuations affect population dynamics in competitive communities has long been debated (Chesson and Huntly, 1997; Ranta *et al.*, 2006). Much of this discussion has focused on whether and how environmental variation enables and maintains species diversity (Hutchinson, 1961; Armstrong and McGehee, 1980; Chesson and Huntly, 1997; Chesson 2000). *Serratia marcescens* and *N. capsulatum* were able to co-exist in the two-species communities throughout the 13-week experiment with a pulsed supply of chemically complex resource. According to theory, the general diversity maintaining mechanisms can be divided into variation-independent (e.g. niche differentiation in the use of food resources) and variation-dependent mechanisms (reviewed by Chesson, 2000). The variation-dependent mechanisms include: (1) the storage effect, where competing species experience spatial or temporal differences in growth rates (Chesson, 1994); and (2) relative non-linearity, where species-specific responses to resource availability differ and the availability of resources fluctuates (Armstrong and McGehee, 1980; Chesson, 2000). Resource availability may vary temporally (as in the pulsed resource environments) and spatially. Variation in resource availability may cause variation in the competitive ranking of the species, an important component of variation-dependent species co-existence (Chesson and Huntly, 1997). Our results on the responses of *N. capsulatum* and *S. marcescens* to different resource concentrations clearly show that the species had different growth responses in low versus high resource concentrations (see Methods). Because species differ in their resource uptake functions, this could enable species co-existence through the relative non-linearity mechanism in a fluctuating resource environment (Armstrong and McGehee, 1980; Chesson, 2000). Bacteria also commonly differ in their metabolic capabilities (Madigan *et al.*, 2010), suggesting that the co-existence could have resulted from between-species differences in the use of different chemical components of the hay extract medium. Thus, both the relative non-linearity and the possible differences in the use of different chemical substances present in the hay extract medium could explain the long-term co-existence of *S. marcescens* and *N. capsulatum* in the pulsed-resource environment. These two mechanisms are apparently not mutually exclusive, and further experiments are needed to test how much these mechanisms contribute to the co-existence of species in this system.

One of our objectives was to compare how community composition and resource pulse amplitude influence the long-term population dynamics. We found that these factors interactively influence both the ecological and the evolutionary dynamics of the system. One indication of this interaction is that in environments where the resource pulse was large, the species ratio changed during the 13-week experiment in favour of *S. marcescens* in the two-species communities. Both species reached larger end population sizes by the end of the resource pulse when grown as monocultures than in two-species communities, indicating that these species competed for resources. During the early phase of the pulse, both species grew faster if the resource pulse was large than if the pulse was small, as expected. However, the species also differed in their response to resource pulse amplitude. The opportunist *S. marcescens* had the shortest time lag before the onset of the rapid growth phase in the large pulse environment. The gleaner *N. capsulatum* had the highest end population sizes in the small resource pulse environment that was characterized by lower resource input and lower outflow mortality during resource renewal. These ecological differences can partly explain the abundance of *S. marcescens* in the two-species communities. Note, however, that *N. capsulatum* did not completely disappear from the two-species communities. Chesson and Huntly (1997) demonstrate that the importance of competitive interactions on co-existence is not diminished by changes in resource availability. They emphasize the importance of intra- versus inter-specific competition in species co-existence: as long as

the competition is stronger between conspecifics than between species, competitive exclusion is unlikely (Chesson and Huntly, 1997; Chesson, 2000). It is possible that in our study system intra-specific competition for resources was stronger than inter-specific competition. In addition, there were evolutionary changes in several traits that most likely contributed to the increasing competitive superiority of *S. marcescens* in the large resource pulse environment. Although mortality decreased in both species, *S. marcescens* in particular was able to evolve towards reduced mortality in the two-species communities experiencing large resource pulses. Moreover, the end population size of *S. marcescens* improved most prominently in the large amplitude resource pulse environment in the two-species communities. Both the low mortality and the ability to maintain high population size during resource scarcity can be used as indicators of a low resource stress resistance (Chesson and Huntly, 1997). Based on the growth strategies of the ancestor strains, we hypothesized that *S. marcescens* would have a competitive advantage during the early phase of the resource pulse, while during the late phase the competitive ranking would change in favour of *N. capsulatum*. Against this expectation, *S. marcescens* was competitively superior also during the late phase of the pulse, especially in the large resource pulse environments. Taken together, our results show that the evolutionary changes in the species with an opportunist growth strategy can potentially change the outcome of competition.

With the accumulation of knowledge on how rapid evolutionary changes affect the life histories and population dynamics of organisms (see review by Hairston *et al.*, 2005), new concepts emerge, such as evolutionary rescue (Bell and Collins, 2008), that emphasize the importance of experiment-based knowledge for ecological and evolutionary dynamics. The interplay of evolutionary changes and ecological interactions can define the long-term persistence of communities in changing environmental conditions. Here, we showed experimentally how periodical fluctuations between generations in resource availability cause evolutionary changes in life-history traits. These changes in turn shape population and community dynamics. The magnitude and the direction of changes depend on both the abiotic and the biotic environment (here the competitive environment). It is known that rapid evolutionary changes may shape the ecological dynamics in systems with a living and evolvable resource and a consumer [predator–prey systems (e.g. Yoshida *et al.*, 2007; Friman *et al.*, 2008)]. Here, we have demonstrated that rapid evolutionary changes occur also in communities within one trophic level. Our results highlight the importance of integrating evolutionary dynamics to the ecological theories of community and population dynamics and the interplay between resource supply and biotic interactions in shaping the evolution of life-history traits.

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REFERENCES

- Armstrong, R.A. and McGehee, R. 1980. Competitive exclusion. *Am. Nat.*, **115**: 151–170.
 Bell, G. and Collins, S. 2008. Adaptation, extinction and global change. *Evol. Appl.*, **1**: 3–16.

- Buckling, A., Brockhurst, M.A., Travisano, M. and Rainey, P.B. 2006. Experimental adaptation to high and low quality environments under different scales of temporal variation. *J. Evol. Biol.*, **20**: 296–300.
- Carrero-Colón, M., Nakatsu, C.H. and Konopka, A. 2006. Effect of nutrient periodicity on microbial community dynamics. *Appl. Environ. Microbiol.*, **72**: 3175–3183.
- Chesson, P. 1994. Multispecies competition in variable environments. *Theor. Pop. Biol.*, **45**: 227–276.
- Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.*, **31**: 343–366.
- Chesson, P. and Huntly, N. 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *Am. Nat.*, **150**: 519–553.
- Chesson, P., Gebauer, R.L.E., Schwinning, S., Huntly, N., Wiegand, K., Ernest, M.S.K. *et al.* 2004. Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia*, **141**: 236–253.
- Ferenci, T. 2001. Hungry bacteria – definition and properties of a nutritional state. *Environ. Microbiol.*, **3**: 605–611.
- Finkel, S.E. 2006. Long-term survival during stationary phase: evolution and the GASP phenotype. *Nature Rev. Microbiol.*, **4**: 113–120.
- Friman, V.-P., Hiltunen, T., Laakso, J. and Kaitala, V. 2008. Availability of prey resources drives evolution of predator–prey interaction. *Proc. R. Soc. Lond. B*, **275**: 1625–1633.
- Gause, G.F. 1934. *The Struggle for Existence*. Baltimore, MD: Williams & Wilkins.
- Goldberg, D. and Novoplansky, A. 1997. On the relative importance of competition in unproductive environments. *J. Ecol.*, **85**: 409–418.
- Grimont, P.A.D. and Grimont, F. 1978. The genus *Serratia*. *Annu. Rev. Microbiol.*, **32**: 221–248.
- Hairston, N.G., Jr., Ellner, S.P., Geber, M.A., Yoshida, T. and Fox, J.A. 2005. Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.*, **8**: 1114–1127.
- Hiltunen, T., Laakso, J., Kaitala, V., Suomalainen, L.-R. and Pekkonen, M. 2008. Temporal variability in detritus resource maintains diversity of bacterial communities. *Acta Oecol.*, **33**: 219–299.
- Holt, R.D. 2008. Theoretical perspectives on resource pulses. *Ecology*, **89**: 671–681.
- Hutchinson, G.E. 1961. The paradox of plankton. *Am. Nat.*, **95**: 137–145.
- Kassen, R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. *J. Evol. Biol.*, **15**: 173–190.
- Krieg, N.R. and Holt, J.G., eds. 1984. *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD: Williams & Wilkins.
- Leifson, E. 1962. The bacterial flora of distilled and stored water. III. New species of the genera *Corynebacterium*, *Flavobacterium*, *Spirillum*, and *Pseudomonas*. *Int. Bull. Bacteriol. Nomencl. Taxon.*, **12**: 161–170.
- Lenski, R.E. and Travisano, M. 1994. Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. *Proc. Natl. Acad. Sci. USA*, **91**: 6808–6814.
- Lenski, R.E., Rose, M.R., Simpson, S.C. and Tadler, S.C. 1991. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *Am. Nat.*, **138**: 1315–1341.
- Levins, R. 1979. Coexistence in a variable environment. *Am. Nat.*, **114**: 765–783.
- Luckinbill, L.S. 1978. *r*- and *K*-selection in experimental populations of *Escherichia coli*. *Science*, **202**: 1201–1203.
- Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. 2010. *Biology of Microorganisms*. London: Pearson.
- McArthur, J.V. 2006. *Microbial Ecology: An Evolutionary Approach*. London: Academic Press, Elsevier.
- Novak, M., Pfeiffer, T., Lenski, R.E., Sauer, U. and Bonhoeffer, S. 2006. Experimental tests for an evolutionary trade-off between growth rate and yield in *E. coli*. *Am. Nat.*, **168**: 242–251.

- Ranta, E., Lundberg, P. and Kaitala, V. 2006. *Ecology of Populations*. Cambridge: Cambridge University Press.
- Reboud, X. and Bell, G. 1996. Experimental evolution in *Chlamydomonas*. III: Evolution of specialist and generalist types that vary in space and time. *Heredity*, **78**: 507–514.
- Roff, D.A. and Fairbairn, D.J. 2007. The evolution of trade-offs: where are we? *J. Evol. Biol.*, **20**: 433–447.
- Roughgarden, J. 1971. Density-dependent natural selection. *Ecology*, **52**: 453–468.
- Rozen, D.E., Nadège, P., de Visser, J.A., Lenski, R.E. and Schneider, D. 2009. Death and cannibalism in a seasonal environment facilitate bacterial coexistence. *Ecol. Lett.*, **12**: 34–44.
- Spencer, C.C., Saxer, G., Travisano, M. and Doebeli, M. 2005. Seasonal resource oscillations maintain diversity in bacterial microcosms. *Evol. Ecol. Res.*, **9**: 775–787.
- Stearns, S.C. 1989. Trade-offs in life-history evolution. *Funct. Ecol.*, **3**: 259–268.
- Takeuchi, M., Hamana, K. and Hiraishi, A. 2001. Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int. J. Syst. Evol. Microbiol.*, **51**: 1405–1417.
- Trosvik, P., Rudi, K., Næs, T., Kohler, A., Chan, K.-S., Jakobsen, K.S. *et al.* 2008. Characterizing mixed microbial population dynamics using time-series analysis. *ISME J.*, **2**: 707–715.
- Vasi, F. and Lenski, R.E. 1999. Ecological strategies and fitness tradeoffs in *Escherichia coli* mutants adapted to prolonged starvation. *J. Genet.*, **78**: 43–49.
- Vasi, F., Travisano, M. and Lenski, R.E. 1994. Long-term experimental evolution in *Escherichia coli*. II. Changes in life-history traits during adaptation to a seasonal environment. *Am. Nat.*, **144**: 432–456.
- Yang, L.H., Bastow, J.L., Spence, K.O. and Wright, A.N. 2008. What can we learn from resource pulses? *Ecology*, **89**: 621–634.
- Yoshida, T., Ellner, S.P., Jones, L.E., Bohannan, B.J., Lenski, R.E. and Hairston, N.G., Jr. 2007. Cryptic population dynamics: rapid evolution masks trophic interactions. *PLoS Biol.*, **5**: 1868–1879.