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# Evolution of bacterial life-history traits is sensitive to community structure

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**Very few studies have experimentally assessed the evolutionary effects of species interactions within the same trophic level. Here we show that when *Serratia marcescens* evolve in multispecies communities, their growth rate exceeds the growth rate of the bacteria that evolved alone, whereas the biomass yield gets lower. In addition to the community effects per se, we found that few species in the communities caused strong effects on *S. marcescens* evolution. The results indicate that evolutionary responses (of a focal species) are different in communities, compared to species evolving alone. Moreover, selection can lead to very different outcomes depending on the community structure. Such context dependencies cast doubt on our ability to predict the course of evolution in the wild, where species often inhabit very different kinds of communities.**

**KEY WORDS:** **Bacteria, competition, experimental evolution, *Serratia marcescens*, species diversity.**

The ecological literature suggests that the function of communities is controlled, not only by their sheer diversity, but also through key species (Hooper et al. 2005). Therefore, community effects, and "dominating and facilitating" species in particular, could play an important role in determining evolutionary responses. Such key species or community-specific effects could greatly affect the generality of evolutionary predictions. However, experimental evidence for how living in multispecies communities shapes evolutionary trajectories is scarce (Turcotte et al. 2012).

The presence of other species could promote the speed of evolution via diversity of interspecies interactions (Powell and Wistrand 1978; Lankau and Strauss 2007), or decrease it due to increased competition, partitioning of resources, niche packing, and smaller population sizes (Falconer and Mackay 1996; Brockhurst et al. 2007; de Mazancourt et al. 2008; Silvertown et al. 2009; Lawrence et al. 2012; Collins 2011). Because competition for resources is expected to increase in communities, species might evolve enhanced use of the most profitable resource (i.e., compete harder). For example, in pulsed resource environments (i.e., where the resources are renewed periodically), the rapid utilization of resources can be attained with elevated growth rate (Holt 2008). Elevated growth rate not only allows for a fast increase in population biomass, but also effectively limits the amount of resource that is available for the competing species. However, within species competition could be stronger because the resource use overlap of similar genotypes may restrict resource use even more compared to between species competition, if different species use resources differently. If this was the case, growth rate would evolve higher if species lived alone than if they evolved in communities. It could also be that inclusive fitness benefits of lower competition with kin would temper growth rate-mediated competition in single-species populations. In other words, the clones could sense conspecifics as kin, rather than competitors, especially if culture conditions force low genetic diversity within species (e.g., Rainey and Travisano 1998; Inglis et al. 2009; Bashey et al. 2012). In addition to growth differences, individuals could evolve higher levels of interference competition due to the competing species. For instance, many bacteria are known to produce toxins that negatively affect the growth of other bacterial species (Riley and Wertz 2002) and also distantly related conspecifics in social evolution context (Lankau and Strauss 2007; Inglis et al. 2009; Bashey et al. 2012). Due to the fact that competition arises by partitioning of scarce






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resources between species, environmental productivity can also influence the evolutionary processes in communities, with more relaxed selection pressure when resources are abundant.

Communities differ in their ecology and therefore their expected evolutionary trajectories could be highly dependent on the presence of particular species. Although community living itself exerts selection pressures, certain species in the community could be especially important. This could be because of pairwise competition, facilitation, or via dominance effects caused by large population sizes of these species (Loreau and Hector 2001; Stinchcombe and Rausher 2001; Friman and Buckling 2012; Lawrence et al. 2012). Thus, the evolutionary effects of the community structure might not be due to evolution in a community per se, but also due to an effect of presence or absence of interacting "key" species.

The role of community structure in shaping ecology and evolution has been acknowledged in the literature (Hooper et al. 2005; Gilman et al. 2010), but long-term studies on how community structure affects evolutionary changes are very limited (TerHorst 2010; Lawrence et al. 2012; Turcotte et al. 2012; Fiegna et al. 2014). Moreover, microbial work with community evolution has concentrated on the presence/absence of bacterial enemies and not on bacteria–bacteria interactions (Bohannan and Lenski 1999; Friman et al. 2008; Gilman et al. 2010; Friman and Buckling 2012; Hiltunen et al. 2014; Zhang et al. 2014). Multispecies experiments that go beyond two species interactions (Lawrence et al. 2012; Fiegna et al. 2014) are a logical continuum to the successful experimental evolution research that has tested many evolutionary hypotheses in simpler systems (Buckling et al. 2009). First, it is important to answer if single-species systems differ from multispecies systems in their evolutionary responses. If living in a community changes evolutionary trajectories, one could ask how realistic are the evolutionary predictions obtained from single-species studies? Second, if a particular community composition or a species in the community alters evolutionary trajectories, are evolutionary changes therefore very context dependent? Such context dependencies cast a doubt on our ability to predict the course of evolution in the wild, where species inhabit very different kinds of communities.

To test the effects of community structure on evolution, we developed a single focal species experimental approach. The focal species, *Serratia marcescens*, was allowed to evolve alone or as a member of 20 different kinds of four-species communities, i.e., *S. marcescens* and three other species drawn from the pool of six laboratory-adapted bacterial species (Table 1). This experiment was conducted in both high and low resource levels, and the evolved growth traits were also measured in high and low resource environments. We hypothesized that evolution in a community with other species would increase *S. marcescens* growth rate, enhancing its speed of resource utilization and strengthening its dominance over competitors. This is a feasible prediction since *S. marcescens* competes with all of the species used in the study in the laboratory (see Electronic supplementary material) and is able to invade and dominate these communities (Ketola et al., unpubl. ms.). Alternatively, if within species competition was the strongest selective force, we would expect that strains that have evolved alone would grow faster. Moreover, we predicted that the effects of competitors on *S. marcescens* growth rate would be more pronounced in scarce resources. The use of several community types (Table 1) allowed us to test which of the following two aspects most affect the evolution of *S. marcescens* traits: living alone versus in community, or absence versus presence of certain key species.

## Materials and Methods

We experimentally manipulated community structure and allowed *S. marcescens* to evolve alone or as a member of 20 different combinations of four-species communities (Table 1). We selected easily culturable, laboratory-adapted species, to minimize problems in identification and culturing. However, it should be noted that although *S. marcescens* can be found in a vast diversity of environments in the wild (Grimont and Grimont 1978), some of the other bacterial species might not encounter each other in natural conditions. Consequently, the evolutionary impact of individual species could be weaker in their natural species assemblages, where extinction is possible or where competition has resulted in the evolution of adaptations that reduce competitive interactions (but see Foster and Bell 2012).

In addition to community manipulation, we conducted the experiment in two resource levels. A single *S. marcescens* clone, grown overnight to high density, seeded all 60 experimental populations: 10 populations of *S. marcescens* alone in low resources, 10 populations of *S. marcescens* alone in high resources, 20 populations of *S. marcescens* with competitors in low resources, and 20 populations of *S. marcescens* with competitors in high resources. The experimental populations and communities were kept in 15 ml centrifuge tubes (Sarstedt, Numbrecht, Germany) containing 5 ml of nutrient broth medium (see below), without shaking in room temperature of about 22°C. Tube caps were kept loose to allow gas exchange. Half of the populations were reared in 100% nutrient broth medium (high resource level; 10 g nutrient broth (Difco, Becton and Dickinson, Sparks, MD) and 1.25 g yeast extract (Difco) autoclaved in 1 L of $dH_2O$ for 20 minutes), and the other half were reared in 25% nutrient broth medium (low resource level; 2.5 g nutrient broth and 0.3125 g yeast extract autoclaved in 1 L of $dH_2O$). Every 3 days, 1 ml of well-mixed population of *S. marcescens* was transferred to a new tube containing 5 ml of fresh resources. In the populations where *S. marcescens* was grown with three competitor species, we transferred 900 μl of

**Table 1.** Different three-species communities in the experiment where *Serratia marcescens* were reared either alone (10 replicate populations) or in any of the 20 different bacterial communities (all possible combinations of six species).

| Community | Species 1 | Species 2 | Species 3 |
|---|---|---|---|
| *1* | *Novosphingobium capsulatum* | *Pseudomonas chlororaphis* | *Pseudomonas putida* |
| *2* | *Novosphingobium capsulatum* | *Pseudomonas chlororaphis* | *Escherichia coli* |
| *3* | *Novosphingobium capsulatum* | *Pseudomonas chlororaphis* | *Enterobacter aerogenes* |
| *4* | *Novosphingobium capsulatum* | *Pseudomonas chlororaphis* | *Leclercia adecarboxylata* |
| *5* | *Novosphingobium capsulatum* | *Pseudomonas putida* | *Escherichia coli* |
| *6* | *Novosphingobium capsulatum* | *Pseudomonas putida* | *Enterobacter aerogenes* |
| *7* | *Novosphingobium capsulatum* | *Pseudomonas putida* | *Leclercia adecarboxylata* |
| *8* | *Novosphingobium capsulatum* | *Escherichia coli* | *Enterobacter aerogenes* |
| *9* | *Novosphingobium capsulatum* | *Escherichia coli* | *Leclercia adecarboxylata* |
| *10* | *Novosphingobium capsulatum* | *Enterobacter aerogenes* | *Leclercia adecarboxylata* |
| *11* | *Pseudomonas chlororaphis* | *Pseudomonas putida* | *Escherichia coli* |
| *12* | *Pseudomonas chlororaphis* | *Pseudomonas putida* | *Enterobacter aerogenes* |
| *13* | *Pseudomonas chlororaphis* | *Pseudomonas putida* | *Leclercia adecarboxylata* |
| *14* | *Pseudomonas chlororaphis* | *Escherichia coli* | *Enterobacter aerogenes* |
| *15* | *Pseudomonas chlororaphis* | *Escherichia coli* | *Leclercia adecarboxylata* |
| *16* | *Pseudomonas chlororaphis* | *Enterobacter aerogenes* | *Leclercia adecarboxylata* |
| *17* | *Pseudomonas putida* | *Escherichia coli* | *Enterobacter aerogenes* |
| *18* | *Pseudomonas putida* | *Escherichia coli* | *Leclercia adecarboxylata* |
| *19* | *Pseudomonas putida* | *Enterobacter aerogenes* | *Leclercia adecarboxylata* |
| *20* | *Escherichia coli* | *Enterobacter aerogenes* | *Leclercia adecarboxylata* |

**This experiment was conducted both in high and low resource concentrations.**

culture to the fresh resources. To ensure that community composition was maintained despite *S. marcescens* being highly competitive against other species, we added 33 μl of overnight culture of each of the three competitor species to the experimental communities at every renewal (Table 1). Thus, in both levels of community treatment the amount of available resources to bacterial growth was the same. The experiment lasted for 1 month.

At the end of the experiment, we isolated six *S. marcescens* clones from every population by dilution plating bacterial samples to Dnase test agar with methyl green (Becton and Dickinson and Company, Sparks, MD). The Dnase activity of *S. marcescens* distinguishes it from the other bacteria used in the experiment, because they all lack Dnase activity after a 24-h incubation at 30°C. After 48 h, *S. marcescens* colonies (which were marked the previous day on the plates, when the halo-effect was clearest) were picked with an inoculation loop and mixed with 500 μl of 80% glycerol and frozen at −80°C. Later, we thawed the clones and transferred 0.5 ml of each culture to 2ml of 100% nutrient broth. After 24 h, 200 μl of culture was mixed with 80% glycerol (1:1) and pipetted into four Bioscreen C® honeycomb well plates (Oy Growth Curves Ab, Ltd., Helsinki, Finland) in a prerandomized order. These clone library plates were frozen at −80°C for later use. This clone library allows an easy onset of measurements of clones in different conditions using a cryo-replication system that allows transferring small amounts of bacteria from frozen clone library to fresh medium without a need to thaw the original samples. When samples are also in randomized order one can use the system very efficiently with a very large number of clones (Duetz et al. 2000; Ketola et al. 2013).

Clones from the library were transferred to fresh measurement resources using a heat-sterilized cryo-replicator. After 24 h, the clones were replicated to new plates containing fresh measurement medium. This overnight culturing is important to remove the glycerol residues from the medium, which otherwise would affect growth. In addition, this incubation allows the strains to acclimate to the same "common garden" culturing conditions before measurements are conducted. We measured growth in two Bioscreen spectrophotometers, one concentration of nutrient broth at a time (25 or 100%). Changes in optical density (OD) caused by bacterial growth were measured at 600 nm for 3 days, at 5-minute intervals. The OD data were used to calculate maximal growth rate and yield with a MATLAB script written by T. Ketola. Briefly, it fits a linear regression slope to 30 time step long sliding window of log-transformed data (the log transformation linearizes the exponential growth phase). The steepest regression slope found from of all fitted windows determines the maximum growth rate. Yield is estimated as the largest average OD over the 30 time-point sliding window.

Treatment effects on growth parameters were tested with mixed models in SPSS (version 20.0, IBM). The mixed model

**Table 2.** Results from the mixed model analysis (REML) testing evolutionary consequences of competitor species, resource levels, and measurement resource levels on growth rate and yield of *Serratia marcescens*.

| ***Growth rate*** | Base model | | | | Best model | | | |
|---|---|---|---|---|---|---|---|---|
| *Source* | *df1* | *df2* | *F* | *Sig.* | *df1* | *df2* | *F* | *Sig.* |
| Intercept | 1 | 693.225 | 1217.393 | **<0.001** | 1 | 671.017 | 1189.031 | **<0.001** |
| Measurement resource (M) | 1 | 710 | 182.531 | **<0.001** | 1 | 706.407 | 175.396 | **<0.001** |
| Resource (R) | 1 | 58.737 | 0.642 | 0.426 | 1 | 56.045 | 0.665 | 0.418 |
| Community (C) | 1 | 59.772 | 5.432 | **0.023** | 1 | 54.379 | 6.851 | **0.011** |
| M × R | 1 | 657.397 | 1.237 | 0.266 | 1 | 657.6 | 1.125 | 0.289 |
| M × C | 1 | 657.323 | 1.178 | 0.278 | 1 | 657.758 | 1.123 | 0.29 |
| R × C | 1 | 56.726 | 7.916 | **0.007** | 1 | 53.831 | 9.258 | **0.004** |
| M × C × R | 1 | 654.932 | 7.871 | **0.005** | 1 | 655.077 | 7.799 | **0.005** |
| Inoculum | 1 | 696.821 | 354.347 | **<0.001** | 1 | 683.444 | 345.618 | **<0.001** |
| Inoculum nested within M | 1 | 709.982 | 108.973 | **<0.001** | 1 | 706.622 | 103.37 | **<0.001** |
| *E. aerogenes* | | | | | 1 | 53.027 | 6.347 | **0.015** |
| *P. chlororaphis* | | | | | 1 | 53.574 | 2.189 | 0.145 |
| *N. capsulatum* | | | | | 1 | 53.06 | 2.931 | 0.093 |
| | *est* | *SE* | *Wald Z* | *sig* | *est* | *SE* | *Wald Z* | *sig* |
| Population | 0.000425 | 0.000218 | 1.947 | 0.052 | 0.000513 | 0.000225 | 2.278 | **0.023** |
| ***Yield*** | Base model | | | | Best model | | | |
| *Source* | *df1* | *df2* | *F* | *Sig.* | *df1* | *df2* | *F* | *Sig.* |
| Intercept | 1 | 699.797 | 257.827 | **<0.001** | 1 | 688.334 | 259.949 | **<0.001** |
| Measurement resource (M) | 1 | 709.54 | 33.356 | **<0.001** | 1 | 707.97 | 30.907 | **<0.001** |
| Resource (R) | 1 | 58.579 | 0.606 | 0.439 | 1 | 56.591 | 0.63 | 0.431 |
| Community (C) | 1 | 59.526 | 1.377 | 0.245 | 1 | 55.219 | 9.143 | **0.004** |
| M × R | 1 | 657.154 | 0.408 | 0.523 | 1 | 657.073 | 0.458 | 0.499 |
| M × C | 1 | 657.069 | 0.001 | 0.977 | 1 | 657.185 | 0 | 0.991 |
| R × C | 1 | 56.719 | 0.54 | 0.466 | 1 | 54.561 | 0.596 | 0.443 |
| M × C × R | 1 | 654.894 | 0.145 | 0.703 | 1 | 654.768 | 0.152 | 0.697 |
| Inoculum | 1 | 702.988 | 16.487 | **<0.001** | 1 | 695.63 | 18.672 | **<0.001** |
| Inoculum nested within M | 1 | 709.345 | 3.302 | **0.07** | 1 | 707.901 | 4.169 | **0.042** |
| *P. chlororaphis* | | | | | 1 | 54.549 | 8.352 | **0.006** |
| *L. adecarboxylata* | | | | | 1 | 53.738 | 3.924 | 0.053 |
| | *est* | *SE* | *Wald Z* | *sig* | *est* | *SE* | *Wald Z* | *sig* |
| Population | 0.000245 | 0.00019 | 1.293 | 0.196 | 0.000357 | 0.0002 | 1.785 | 0.074 |

contained fixed effects of community treatment (evolved alone or with other species), resource treatment (evolved in high or low resources), measurement resource (measured in high or low resources), and all three- and two-way interactions. To control for different population sizes at the beginning of the measurements we used the mean of the first three OD measurements (i.e., inoculum) as a fixed covariate. In addition, we fitted measurement resource-specific covariates for inoculum, by nesting the inoculum within the level of measurement concentration, since different nutrient broth concentrations lead to different effects of inoculum on growth rate. To control for the nonindependency of observations arising from six extracted clones from the same population, we fitted identity of the population as a random factor (nested within evolutionary treatments). The above-mentioned test is the so-called base model on top of which we added the effects of single species and tested if the presence or absence of individual species affected the growth rate or yield. Evolutionary effects of competitor species on growth parameters were tested separately, in pairs, and three species together, with all other above-mentioned predictors in the model (i.e., base model). Note that all possible combinations of species exist in our dataset. Estimates of individual species' effects were resolved by model averaging methodology to obtain averaged effect of single species on *S. marcescens* evolution over different models (Burnham and Anderson 2002; Symonds and Moussalli 2011).We resolved the best models with maximum likelihood with AIC and used

restricted maximum likelihood for obtaining estimates for the best models. We present the results from these best models (see Results) and when there is deviation in biological interpretations between the "best" and the base model (model fitted without species absence presence data, Table 2) the estimates from the base model are also shown. To explore if the resource use overlapped between species we performed separate competition experiments (Fiegna et al. 2014; Foster and Bell 2012).

## Results

The clones evolved a higher growth rate in the presence of competitor species (estimated marginal mean: 0.961, standard error [SE]: 0.006) compared to single-species cultures (0.924, SE: 0.013; Table 2, Fig. 1A). If clones had evolved in low resources, the growth rate was clearly lower in clones that had evolved alone (0.908, SE: 0.015) than if they had evolved in communities (0.970, SE: 0.007, $P < 0.001$, Fig. 1A). However, such difference was not visible if strains had evolved in high resources ($P = 0.522$, Fig. 1A, evolved alone (0.941, SE: 0.015), evolved in communities (0.951, SE: 0.008)). High measurement resources masked the effect of community living, if the clones had also evolved in high resources (alone: 1.157, SE: 0.019 in community: 1.139, SE: 0.011, $P = 0.385$). In all the other factor combinations, living in a community always resulted in a significantly higher growth rate (see Fig. 1A). Measurements done in high resource concentrations (1.141, SE: 0.010), unsurprisingly, found larger growth rates than measurements done at lower resource concentrations (0.744, SE: 0.008, $P < 0.001$; Table 2, Fig. 1A). When the inoculum (i.e., the amount of bacterial biomass at the onset of measurement) was larger, the growth rate was smaller ($b = -15.976$, SE: 1.05, $P = 0.001$). Moreover, the effect of inoculum on growth rate was less profound in low than in high measurement resource concentrations (difference in slopes: 10.99, SE: 1.08, $t = 10.164$, $P = 0.001$). Although the best model contained the absence/presence information of *Enterobacter aerogenes, Pseudomonas chlororaphis, and Novosphingobium capsulatum*, there were clearly competing models that yielded equally good fit to the data ($\Delta AIC < 2$; ESM Table 1). Despite a fair level of uncertainty in the model selection, it was clear that the model without any species absence/presence information (i.e., the base model) had relatively low explanatory power ($\Delta AIC = 7.67$; ESM Table 1). Thus, the results are presented based on "the best model." Because of the model selection uncertainty, the estimates for single-species effects on growth rate were derived by model averaging (Burnham and Anderson 2002; Symonds and Moussalli 2011). Based on these model-averaged estimates it was evident that *E. aerogenes* increased the growth rate (Fig. 2). Although the best model (smallest AIC) also contained the effect of *N. capsulatum* and *P. chlororaphis,* their effects on growth were not clear enough over all of the fitted models (i.e., 95% confidence interval overlapped zero; Fig. 2).

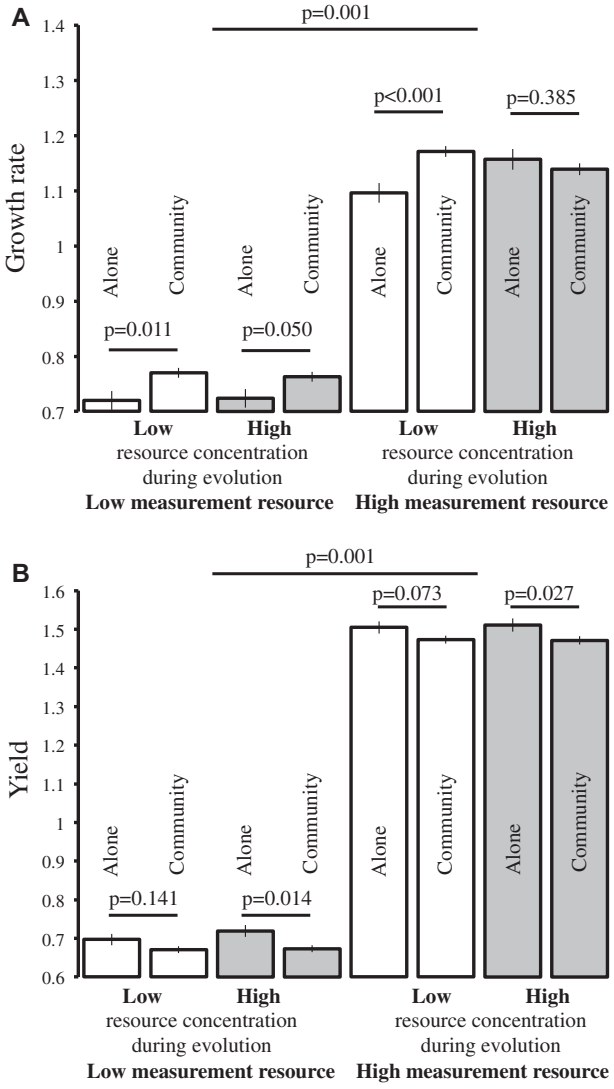


**Figure 1. Results of a mixed model testing growth rate ($OD_{600\ nm}\ h^{-1}$) (A) and yield ($OD_{600\ nm}$) (B) differences of *Serratia marcescens* clones that had evolved alone or in multispecies communities, in high or low resource concentrations and that have been measured in high or low resource concentrations. Depicted means for treatment levels corresponds to the estimated marginal means and standard errors from the model detailed in Table 2.**

The competitors also affected biomass yield, which was lower (est: 1.072, SE: 0.006) than if the clones had evolved alone (est: 1.108 SE: 0.011, Table 2). However, this result was sensitive to absence/presence information of species in the model. In the base model without absence/presence data, bacterial yield did not differ between clones that had evolved alone (1.084, SE: 0.008) and in communities (1.072, SE: 0.006). Otherwise, biological interpretations did not change between the base and the best models. In addition, a larger inoculum was associated

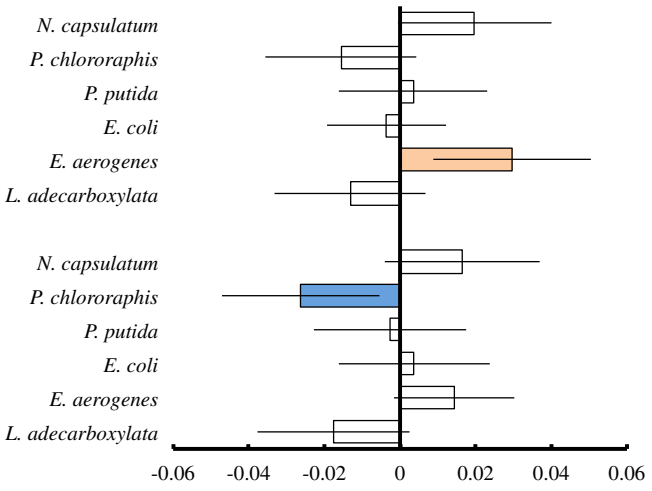


**Figure 2. Effects of the existence of particular competitor species in communities on *Serratia marcescens* growth and yield. Positive values indicate that the presence of the species increased *Serratia marcescens* trait values. Values indicate model averaged difference between estimated marginal means for growth rates ($OD_{600\ nm}\ h^{-1}$) and yield ($OD_{600\ nm}$) of clones evolved in communities with or without particular species. Orange and blue bars represent statistically significant increase and decrease of traits, respectively. Estimated marginal means were obtained across all fitted models (ESM Table 1).**

with a larger yield (b = 3.11, SE = 1.02, $P = 0.002$). The best model also contained the absence/presence information of *Lecleria adecarboxylata* and *P. chlororaphis*. However, based on model averaging we found that *L. adecarboxylata* was the only species that affected *S. marcescens* yield (Fig. 2).

## Discussion

High growth rate, and hence rapid exploitation of resources, is expected to be an important trait in both, within- and between-species competition. However, our results suggest that selection for high growth rate was stronger in communities than in monocultures. In a community with competitor species, fast utilization of resources seems to be paramount, not only for increasing population size, but also for diverting resources away from other species (see also TerHorst 2010 for similar results).

Interestingly, the increase in growth rate was more pronounced if strains had evolved in low resources (Table 2). It seems that the fewer resources there are available due to competition and environmental conditions, the stronger the selection pressure for fast growth. In nutrient-limited conditions, fast utilization of short pulses of higher resource levels could bring a disproportionally large competitive advantage in comparison to situations where the resources are more abundant. However, this result seems to contradict previous findings in plants, where more productive environments caused stronger selection on traits (Stanton et al. 2004). While growth rate increased, maximum yield decreased due to the presence of competitor species. The lowered yield could be an indirect consequence of selection on growth rate as studies frequently find negative correlations between yield and growth rate (Velicer and Lenski 1999; Novak et al. 2006). This supports the theoretical predictions of the metabolic rate versus metabolic efficiency trade-off in microbial systems (Pfeiffer et al. 2001; Frank 2010).

In contrast to our one focal species approach, Lawrence et al. (2012) followed several species simultaneously and found that multiple species evolved to have slower growth in communities than in monocultures. Only a single species was found to evolve a higher growth rate in polycultures than in monocultures. In addition to differences in experimental setups, one potential explanation for this discrepancy could be that we have concentrated on the evolutionary changes of the dominant species, without considering the evolutionary responses of the subordinates. Subordinates might have had a low initial growth rate, and lack competitive ability, and thus might only have been able to respond by reducing competition altogether (Brown and Wilson 1956; Schluter and McPhail 1992; Pekkonen et al. 2011; Lawrence et al. 2012; Pekkonen and Laakso 2012). For example, three out of the four species in Lawrence et al.'s study were found to evolve adaptations for avoiding competition, when they evolved in polyculture (Lawrence et al. 2012).

Our system was designed to prevent extinctions, which can occur rapidly in simple microcosms, and to maintain the full effect of competition in the community treatments. To do so, we regularly supplemented communities with the other (subordinate) species from frozen ancestor stocks, and therefore following their evolutionary changes was not meaningful. Although the total species supplementation corresponded to only 10% of biomass in renewal, it could be that ancestral nonevolved clones affected coevolution, extinction dynamics, and population dynamics (Hiltunen and Becks 2014), and thus the generality of our findings. Our resource renewal scheme could also be criticized because of different amounts of freely available resources in different treatments, which could affect life-history trait evolution. In our experimental design, care was taken to allow the whole community (or *S. marcescens* alone) similar chances for growing. This could mean that for *S. marcescens* grown alone, more resources would be available than when grown in communities. However, it is unlikely that our results on evolutionary differences in yield and growth rate would directly reflect resource levels, as the resource manipulation treatment (quadrupled amounts of resources) failed to cause a main effect on either of the traits (Tables 1 and 2). Thus, the results suggest that life-history evolution in communities is more driven by interspecific competitive interactions than overall resource abundance in the system.

Furthermore, we found that while the presence of competitors in general led to increased growth rate and lowered yield, both traits were also sensitive to presence of certain species. Growth rate was clearly increased by presence of *E. aerogenes*, and yield was lower if communities contained *P. chlororaphis*. Note that effects of these species on growth and yield were confirmed over all of the fitted models using model averaging (Burnham and Anderson 2002; Symonds and Moussalli 2011). It is also noteworthy that in the best-ranked model, the effect sizes (Cohen's d, based on *F*-statistics from Table 2) for community effects on growth rate (0.717) closely matched the effect sizes for the presence of *E. aerogenes* (0.689). Similarly, for yield the effect size for the presence of *P. chlororaphis* (0.792) is closely comparable to the effect size for community treatment (0.828). Thus, single "key species" caused almost as large an effect on evolutionary changes as community living itself. Particularly interesting is the observation that without the information of species' absence or presence, the evolutionary effect of community on yield was not detected (Table 2). Thus, it seems that evolutionary consequences of community living can be overwhelmed by few species that happen to be part of that particular community.

We acknowledge that a myriad of microbial competition mechanisms, spanning from resource competition (Foster and Bell 2012) and cross-feeding adaptations (Lawrence et al. 2012; Pekkonen and Laakso 2012) to toxins and phages used as warfare (Riley and Wertz 2002; Bossi et al. 2003) could be behind these phenomena. Although the exact mechanisms for the evolutionary responses that we found in *S. marcescens* against competing bacterial species are still unknown, it is clear that evolution in communities can be strongly affected by a few key species. Previous literature recognizes that competitive effects between species might not be direct, but rather caused indirectly by some other species (Holt 1977). For example, a shared predator, competitor, or facilitator could cause this. If that was the case, statistical effects of a single species would be sensitive to the absence and presence of such species in the statistical model. However, all estimates from species that exerted strong effect on *S. marcescens* were obtained over all possible species compositions with model averaging (Burnham and Anderson 2002; Symonds and Moussalli 2011).

We found that community living selected for increased competitive ability, and lowered yield in comparison to those *S. marcescens* clones that had evolved alone. This result indicates that communities act as a strong selective force and thus change species performance at evolutionary time scales. The most novel finding is that several key species were able to influence evolution almost as much as community living itself. Thus, evolution in a community can be highly dependent on the community structure. Such effects could hinder the accuracy of evolutionary predictions in the wild where evolution always occurs in multispecies communities.

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## DATA ARCHIVING

Data is archived in Dryad doi:10.5061/dryad.mq328.

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## *Supporting Information*

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**ESM Table 1.** Fitted models ranked by their AIC values (smaller is better) and their fit parameters.