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LETTER

The adaptive response of a natural microbial population to carbon- and nitrogen-limitation

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Abstract

The majority of experiments investigating the population and community level consequences of altered carbon and nitrogen availability implicitly assume a fixed range of ability to obtain and process these nutrients. This may be fair when considering long-lived organisms, however, short-lived organisms (e.g. microbes) will pass through many more generations in the same time period providing scope for evolutionary adaptation. Using isolates of a natural fungal population we demonstrate significant adaptation to carbon-limitation, but not to nitrogen-limitation, over a modest number of generations. However, adaptation comes at a cost: those adapted to carbon-limitation are significantly worse competitors for limited nitrogen when compared with their ancestors. The potential for adaptation (and correlated cost) we demonstrate with our model system highlights the need to consider evolutionary adaptation when interpreting responses of populations, communities and ecosystems to treatments that manipulate, either directly or indirectly, resource availability.

Keywords

Adaptation, chemostat, evolution, fitness, fungi, heterotrophs, resource availability, trade-off, yeast.

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INTRODUCTION

Resource availability is one of the dominant structuring forces of communities and ecosystems (van Breemen & Finzi 1998; Elser *et al.* 2000; Wardle 2002). Changes in resource availability occur across short and long temporal scales (e.g. seasonality vs. succession; Connell & Slatyer 1977; Jaeger *et al.* 1999) and in response to environmental disturbance (e.g. land-use change and elevated nitrogen deposition; Asner *et al.* 1997; Nadelhoffer *et al.* 1999). Although responses to changes in the absolute and/or relative availability of carbon and nitrogen may be one of the best researched areas of altered resource availability (Vitousek *et al.* 1997; Körner 2000), ecologists mostly think of populations and communities responding within a fixed range of ability to obtain and process these nutrients (but see Tessier & Woodruff 2002). Only occasionally is the contribution of evolutionary adaptation to the magnitude of measured treatment responses considered (e.g. Bazzaz *et al.* 1995; Ward *et al.* 2000). In comparison with organisms such as earthworms, annual plants, and oak trees, microbes experience many more generations in a given time period. A greater number of generations provides marked potential for genetic adaptation to altered resource availability. Such adaptive changes may alter nutrient dynamics within, and thus the structure of, communities within relatively short timescales (years to decades).

Here, we measure the adaptive evolutionary response of natural fungal isolates to carbon- and nitrogen-limitation across relatively few generations within a model experimental system. Our primary objective is to ascertain the potential for natural microbial populations to adapt to altered carbon and nitrogen availability. We might not expect to observe optimally adapted organisms in nature given that environmental selection pressures vary across time (Cody 1974). Rather, we expect populations to track moving fitness optima and would predict adaptation by any population subjected to a consistent selection pressure. The question is not so much whether a population will adapt but rather how quickly, to what extent and at what cost. Domesticated microbes (*Escherichia coli* and *Saccharomyces cerevisiae*) can adapt to carbon-limited environments (Paquin & Adams 1983; Lenski *et al.* 1991). The ecological relevance of these findings is questionable given that these strains have been perpetually propagated upon laboratory media: their adaptive responses may not reflect those of their natural

counterparts. However, adaptation of *E. coli* to specific carbon substrates has been shown to decrease its performance on alternative and substitutable carbon substrates (Cooper & Lenski 2000). Such an adaptive trade-off is not theoretically predicted under adaptation to different essential resources (e.g. carbon cannot be substituted for nitrogen and *vice-versa*; Abrams 1987). As far as we are aware, this is the first empirical study to measure the adaptive response of a natural microbial population to altered carbon and nitrogen availability and, if adaptation does occur, determine whether there is a trade-off associated with utilization of the alternate essential resource.

MATERIAL AND METHODS

Saccharomyces paradoxus Bachinskaya is a unicellular yeast found widely in terrestrial ecosystems (Naumov *et al.* 1992, 1998). Seven isolates of these fungi, derived from the bark of oak trees (*Quercus robur* L.) found in Silwood Park, UK (51°22′N, 00°37′W), were used in this study. Isolation and identification details are described in Johnson *et al.* (2003).

We used replicated chemostats (Infors HT, Reigate, UK) to propagate these isolates for 350 generations under tightly controlled resource limitation (Dykhuizen & Hartl 1983). We established each isolate in two independent chemostat vessels and provided them with substrates necessary for growth (yeast nitrogen base 1.7 g L^{-1}, Sigma no. Y1251): one vessel was limited for carbon (supplemented with 0.4 g L^{-1} glucose and 5 g L^{-1} ammonium sulphate), and the other limited for nitrogen (supplemented with 0.025 g L^{-1} ammonium sulphate and 20 g L^{-1}glucose). Preliminary tests proved these carbon and nitrogen concentrations to be limiting to growth. We grew the isolates simultaneously at 30 °C, shaking with independent flask aeration and each isolate passed through one generation approximately every 3.15 h (dilution rate averaged 0.22 h^{-1}). We term the isolates used to establish our experimental cultures the *ancestral* isolates; they were not exposed to laboratory conditions prior to our experiment except during isolation. Those exposed to 350 generations of nutrient limitation we term *derived* isolates. All isolates were stored in 15% v/v glycerol at −80 °C when not being grown.

Each experimental culture was founded from a single cell and thus, at the experiment start, every cell within an individual culture was genetically identical; genetic variability existed only between replicates (different isolates). Any change in the ability of a specific isolate to grow and reproduce under the defined experimental conditions over the course of 350 generations must have occurred through selection for *de novo* beneficial mutations which confer a greater competitive ability for the single limiting nutrient. We tested for change in competitive ability (fitness) of these isolates by directly competing the ancestral and derived isolates with a closely related *S. cerevisiae* 'reference' strain. Fitness was estimated for each isolate under carbon- and under nitrogen-limitation: the fitness of the derived isolates was estimated for both the nutrient limitation under which they were derived (i.e. in the environment of selection) and the alternate environment (e.g. those derived under carbon-limitation were competed under nitrogen-limitation). This approach enabled us to determine whether any change in fitness under the environment of selection correlated, either positively or negatively, with any change in fitness under the alternate nutrient limitation, i.e. to test for an evolutionary trade-off.

Fitness estimation began by mixing the isolates with the reference strain in roughly equal proportions and then assaying the change in frequency of each competitor over time when grown under experimental conditions. If both are competitively equal then their frequencies will not vary, however, if one is competitively superior it will begin to displace the other. To accurately measure the frequency of each competitor at the beginning and end of each competition a sample of the mixed population was first plated onto YPD (1% yeast extract, 2% peptone, 2% glucose) agar and allowed to form individual colonies: here both competitors can grow. Differentiation between competitors was easily achieved as the reference strain is genetically marked with G418 toxin (Sigma no. G9516) resistance (genotype: α/a, *ho/ho*, *ura3*Δ/*ura3*Δ, *spo11*Δ/*spo11*Δ, *spo13*Δ :: kanMX4/*spo13*Δ :: kanMX4). All colonies were then transferred (replica plated) to YPD media that contained the G418 toxin: here only the reference strain can grow (Wach *et al.* 1998). The frequency of each competitor may be calculated by comparing the number of colonies on each type of plate. Fitness estimates are calculated from a regression of the change in log ratio of the frequency of the two competitors across the competition (here 20 generations on average). The reference strain was constructed using PCR based gene targeting (Wach *et al.* 1998) and standard selection and mating techniques (Burke *et al.* 2000).

To investigate the mechanistic basis of any fitness changes we measured the total biomass, and its carbon and nitrogen content, of the ancestral and derived isolates. Culture samples (25 mL) were taken from the chemostat at the start and end of the 350 generation experimental run. Cells were pelleted, washed three times with sterile distilled water, dried at 40 °C and then weighed. Carbon and nitrogen concentrations were determined using a Roboprep elemental analyser (PDZ Europa, Cheshire, UK).

Analysis of variance was used to investigate whether fitness differed significantly between the ancestral and derived isolates. A full interaction linear model was used with two fixed, discrete factors (evolutionary state and

environment), and isolate identity was included as a non-interacting factor. Percentage carbon and nitrogen values were arcsine square-root transformed, and the amounts sequestered in the ancestral and derived culture biomass were obtained by multiplying biomass values by proportion carbon and nitrogen values. These data were evaluated using *t*-tests.

RESULTS

On testing for adaptation to the environment of selection we found that there was a significant interaction between evolutionary state (ancestral or derived) and environment ($F_{1,18} = 5.8$, $P < 0.05$). Further analysis demonstrated that isolates derived under carbon-limitation were on average 8% fitter than their ancestors ($F_{1,6} = 30$, $P < 0.01$), whereas fitness of those derived under nitrogen-limitation did not change significantly ($F_{1,6} = 0.81$, $P > 0.05$; Fig. 1). Next, we compared the fitness of the ancestral isolates with the fitness of the derived isolates when competed under the alternate environment to which they were derived, to establish whether an evolutionary trade-off existed. Isolates derived under carbon-limitation were significantly less fit ($F_{1,6} = 8.9$, $P < 0.05$) than their ancestors when competed under nitrogen-limitation (Fig. 1). In contrast, the fitness of the isolates derived under nitrogen-limitation did not change significantly ($F_{1,6} = 1.9$, $P > 0.05$) when placed under carbon-limitation (Fig. 1).

Given a constant carbon supply, the mechanisms which enabled the isolates derived under carbon-limitation to out-compete their ancestors may have reasonably involved: (a) an increase in ability to scavenge carbon from the environment; and/or (b) a decrease in the biomass carbon concentration required to reproduce. There was a slight increase in cellular carbon concentration ($P = 0.069$) and a significant increase in biomass ($P < 0.05$), meaning the total amount of carbon sequestered by the derived isolates was significantly greater ($P < 0.01$; Table 1) when compared with their ancestors. Together these data support the first mechanism and refute the second. The possibility that derived cells simply replicated when smaller than ancestral cells was not supported by the measured culture densities or microscopic observations (data not shown) performed over the course of the experiment.

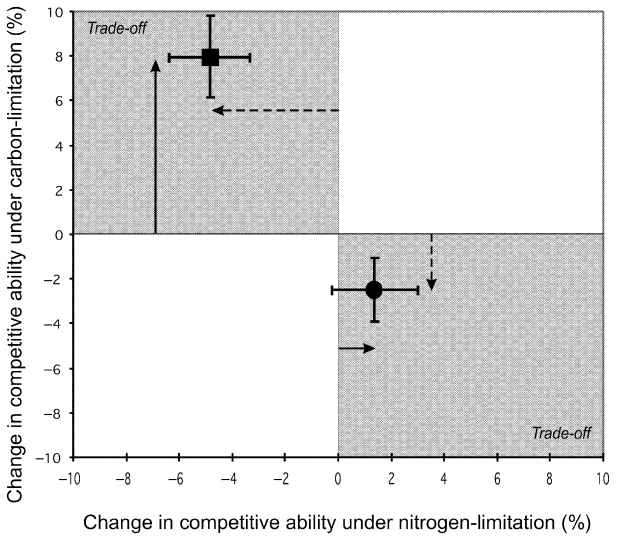

Figure 1 Change in fitness of the isolates derived under carbon-limitation (square) and under nitrogen-limitation (circle), when competed under the environment of selection and under the alternate environment. Solid arrows indicate fitness change in the environment of selection and dashed arrows in the alternate environment. Adaptive trade-offs, i.e. when adaptation to one resource is correlated with a decrease in adaptation to the alternate resource, are marked by the shaded areas. Isolates derived under carbon-limitation displayed significant adaptation and a trade-off ($P < 0.05$), whereas those derived under nitrogen-limitation did not ($P > 0.05$). Values are mean ± 1 SE ($n = 7$).

DISCUSSION

Our primary objective was to ascertain the potential for natural microbial populations to adapt to altered carbon and nitrogen availability. For this reason, we achieved replication through different isolates because we were interested in

Table 1 Biomass, carbon (C) and nitrogen (N) values for the ancestral and derived *S. paradoxus* isolate cultures grown under the two nutrient limitations

Environment of growth	Evolutionary status	Biomass (mg L^{-1})	C (% w/w)	N (% w/w)	Limiting nutrient sequestered (mg L^{-1})
C-limited	Ancestor	155.62 ± 7.419	46.75 ± 0.284	8.65 ± 0.106	72.77 ± 3.574
	Derived	177.77 ± 5.478	47.26 ± 0.245	8.57 ± 0.171	83.98 ± 2.392
N-limited	Ancestor	70.53 ± 8.891	47.85 ± 0.617	5.16 ± 0.139	3.65 ± 0.492
	Derived	96.85 ± 5.687	48.04 ± 0.478	4.68 ± 0.252	4.68 ± 0.194

The isolates derived under carbon-limitation sequestered significantly more carbon than their ancestors ($P < 0.01$). Values are mean ± 1 SE ($n = 7$).

the mean response of a field population (genetic-focussed experiments replicate single isolates). We found that natural fungal isolates were able to genetically-adapt to carbon-limitation in a relatively short-time period but that this adaptation left them poorer competitors under nitrogen-limitation. How might such changes affect resource competition between organisms, a process that directly impacts the functional properties of ecosystems (Loreau 1998)? That ability to scavenge carbon from the environment seems the most likely adaptive change to explain the increased fitness we observed has implications for carbon-resource competition between heterotrophic organisms both within and between populations. Further, that these isolates derived under carbon-limitation were poorer competitors for nitrogen may suggest, for example, that plants will be favoured over microbes in competition for nitrogen in environments where carbon-limitation acts selectively on microbial populations. Such changes will modify local resource distributions, a process that Laland *et al.* (1999) show will significantly alter both ecological and evolutionary patterns within ecosystems. Our data suggest that in environments where resource availability fluctuates over time short-lived organisms will faithfully genetically track changes in nutrient availability although this may have hidden costs; however, longer-lived organisms may be 'buffered' against any costs as their slower rate of genetic adaptation (purely because of generation time) will also mean the slower manifestation of any trade-off. There is a need to test the impacts of such evolutionary-mediated changes on ecosystem development using empirical studies in addition to mathematical models (Loreau 1998; Laland *et al.* 1999).

We can only speculate as to why the isolates subjected to nitrogen-limitation did not significantly increase in fitness while those derived under carbon-limitation did. Presumably selection does little for populations close to their adaptive optimum for a particular trait because, although mutation produces variation, there is little to be improved upon (Cody 1974). Thus, we hypothesize that the ancestral isolates were more adapted to nitrogen- than carbon-limitation, and that this reflects the recent selection pressures experienced by our yeast in the field (i.e. nitrogen was generally more limiting than carbon).

It is hard to determine the generation time of a microorganism in the natural environment (Brock 1971). Population expansion could be rapid under favourable conditions but this expansion is likely to be ephemeral (Clarholm 1994). We have no *S. paradoxus* natural division times but based on other microbial observations it is likely that 350 generations may be achieved within a year to a decade (Brock 1971). Although our experiment falls into the category that uses model systems to test for the existence of phenomena that are hypothesized to operate in the natural environment (Naeem 2001), these timescales are pertinent to many ecological field experiments. We provide empirical evidence that a subset of a natural fungal population, widely found in terrestrial environments, can adapt to carbon-limitation within relatively few generations and that in doing so, becomes less fit when competing for nitrogen. This potential is not explicitly recognized in the majority of studies that report the effects of manipulated or natural changes in carbon and nitrogen availability on ecosystem processes. All other things being equal, the rate of evolution of a species will be a function of generation time. This implies that short lived organisms will adapt more rapidly to changing environmental conditions. No organism will show a fixed response to changes in nutrient availability, but microbes may be quicker to evolve a more competitive edge.

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