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The biogeographical distribution of closely related freshwater sediment bacteria is determined by environmental selection.

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Abstract

The role of environmental selection in governing the structure of communities of freshwater sulfur bacteria (*Achromatium* spp.) was experimentally tested by mixing sediments from two geographically separated lakes (Rydal Water and Hell Kettles) that harboured *Achromatium* spp.. Community profiles of *Achromatium* spp. in sediment microcosms at day 0 and after 60 days were compared to determine whether initial *Achromatium* community composition or, subsequent selection by the sediment environment had greater influence in dictating the final *Achromatium* community structure. It was found that *Achromatium* spp. from the Hell Kettles community became established in mixed sediments at the expense of members of the Rydal Water community. This selection for the Hell Kettles *Achromatium* community was more pronounced when sediment composition was manipulated to resemble Hell Kettles sediments. Our findings definitively demonstrate that environmental selection is the primary determinant of *Achromatium* community structure in these sediments.

Introduction

It has been argued that because of their small size and high rates of dispersal, microorganisms have a global distribution and therefore microbial taxa do not exhibit biogeographical patterns and are ubiquitous (Baas-Becking 1934). The extent, to which this is true, is a matter of considerable debate (Bell *et al.* 2005b, Fenchel and Finlay 2005, Whitfield 2005) and has profound implications for the extent of global microbial diversity, its conservation and its exploitation. The consequence of ubiquitous dispersal and colonization would be the mixing of all species across all continents and none of the regional differences that are familiar in the ecology of larger organisms e.g. the occurrence of marsupials only in Australia and South America. There is evidence to support the notion of ubiquity of some microbial eukaryotes (Finlay 2002) but equally, there is compelling evidence of restricted distribution, species area relationships and endemism, in microbial communities and populations (Bell *et al* 2005a, Cho and Tiedje 2000, Green *et al* 2004, Horner-Devine *et al.* 2004, Oda *et al.* 2003, Papke *et al.* 2003, Reche *et al.* 2005, van der Gast *et al.* 2005, Whitaker *et al.* 2003). To date claims for bacterial endemism have been based on the observed geographical distribution of particular bacterial taxa correlated with their genetic divergence and measured environmental variables (Hughes Martiny *et al.* 2006). These observational case studies are a powerful means of developing theoretical constructs to explain the apparently endemic distribution of some microbial taxa. However, in addition to true endemism there are alternative ecological explanations for patterns that suggest the occurrence of endemic bacterial communities (Rauch and Bar-Yam 2004) and there is a need for experimental testing of theories of biogeography. This was highlighted recently as an important but neglected area of ecological research (Thorpe 2005). One of the major limitations to

assessing apparently endemic distribution patterns in bacterial communities is our poor knowledge of habitat heterogeneity and the selection pressure this imposes on bacteria (Baas-Becking 1934, Horner-Devine *et al.* 2004).

Achromatium are large uncultured sulfur-oxidising bacteria found in freshwater sediments (Gray and Head 1999, Head *et al.* 2000). Although *Achromatium* are relatively large compared to most bacteria, their size is fairly typical of many eukaryotic microorganisms and well below the size limitation proposed to be permissive for effective dispersal by agents such as wind, animals and birds (Finlay 2002). As such these organisms represent a useful model group for studying the ecological mechanisms dictating the distribution of microbial diversity. *Achromatium* species, form a coherent phylogenetic group within the *Gammaproteobacteria* and share distinct characteristics (cell size and shape, presence of calcite and sulfur inclusions (Gray *et al.* 1999a, Gray *et al.* 1999b)) which indicate that members of the genus are functionally similar. Most communities of *Achromatium* spp. studied in any detail occur in shallow, organic carbon and iron rich freshwater sediments at circumneutral pH where concentrations of dissolved sulfide are below detection limits. These low sulfide conditions are unlike those typically encountered in environments which harbour other giant sulfur bacteria, where, dissolved sulfide is abundant. As a result *Achromatium* cannot form conspicuous mats and cannot rely on the diffusive flux of free sulfide as do other giant sulfur bacteria. Instead they are distributed throughout the zone of sulfate reduction and may even utilise other reduced sulfur resources (Gray *et al.* 1997, Gray and Head 1999, Head *et al.* 2000). *Achromatium* communities comprise coexisting species some of which exploit different redox-defined niches as a basis for coexistence (Gray *et al.* 2004). A survey of the geographical distribution of *Achromatium* in lake sediments in the North of

England and Germany has indicated that individual lakes are dominated by different genetically distinct *Achromatium* spp. (Gray *et al.* 1999a). Of these freshwater lakes, two (Rydal Water and Hell Kettles) are located in the North of England and are separated by approximately 60 miles.

Purely observational data on the geographical distribution of *Achromatium* species in Rydal Water and Hell Kettles (Gray *et al.* 1999a) suggesting that each community comprised endemic *Achromatium* species can be interpreted in three ways, illustrating the difficulty in interpreting the ecological significance of purely observational data. Firstly the lakes may have been colonized by a single ancestral *Achromatium* sp. which following geographical separation diverged by adaptive radiation (Rainey and Travisano 1998) to produce the distinct communities of *Achromatium* spp. observed in each lake today (*i.e.* true endemism). Alternatively, the sites are geographically remote with respect to bacterial migration (*i.e.* island-like) (MacArthur and Wilson 1967), and their community composition is a result of restricted dispersal of *Achromatium* spp. and under-saturation with respect to global *Achromatium* diversity. Here it would follow, that regardless of the existence of truly endemic *Achromatium* species, not all possible or best adapted *Achromatium* species have dispersed to every lake, a well known phenomenon in the ecology of island communities which leads to species impoverishment in insular communities (Begon *et al.* 1996). Finally the lakes have been repeatedly, seeded with a wide diversity of *Achromatium* spp. and the predominant *Achromatium* spp. observed are a consequence of selection of those most fit for the prevailing environmental conditions (Bass-Becking 1934, Begon *et al.* 1996). We have used *Achromatium*-bearing sediments from Rydal Water and Hell Kettles to experimentally test which of these mechanisms most likely explains the observed geographical distribution of

Achromatium spp. The experiment was designed to simulate the dispersal of new organisms to a previously un-colonized location.

Materials and methods

Sampling and experimental setup

Grab samples of sediment containing *Achromatium* cells were obtained from a wetland area on the margins of Rydal Water, Cumbria, UK (54°219N, 2°519W) and Hell Kettles, located south of Darlington, County Durham, UK (54°299N, 1°339W) (Gray *et al.* 1999a). Sediments and samples of overlying water (5 cm^3 sediment with 5 cm^3 water) from Rydal Water (RY) and Hell Kettles (HK) were mixed in sterile test tubes (30 ml capacity) and closed with sterile cotton wool. Sediments were mixed at 3 different ratios (Rydal Water volume %: Hell Kettles volume %) 90:10, 75:25, 50:50. In addition unmixed sediment microcosms i.e. those containing pure sediment from one site or the other, were prepared for comparison. A set of microcosms was prepared and immediately sacrificed as time zero controls. In these microcosms each of the mixed sediments initially contained the accumulated diversity of the parent sediments. This experimental strategy increased the diversity of the *Achromatium* community, but, did not increase the population size above the notional carrying capacity of the artificially constructed environments. A duplicate set of sediment microcosms was incubated at 10^oC for 60 days.

Achromatium cell counts and DGGE analysis of Achromatium community structure

Prior to cell extraction for DGGE analysis of *Achromatium* community composition an aliquot of well mixed sediment (100 µl) was removed and made up to 1 ml for the determination of total *Achromatium* cell counts using a Sedgwick Rafter counting chamber (Gray *et al.* 1997). Changes in *Achromatium* community composition over time in the experimental microcosms were assessed by comparison of DGGE profiles of PCR-amplified *Achromatium* 16S rRNA gene fragments. Sediments from experimental microcosms were sequentially filtered through 100 and 64 µm nylon meshes to remove larger sediments particles. Crude cell suspensions obtained in this way were purified as described previously (de Boer *et al.* 1971, Head *et al.* 1996) and were used for DNA extraction. Many *Achromatium* spp. precipitate intracellular calcium carbonate a unique property which makes them very dense and amenable to the gravity based separation procedure. All of the experimental microcosms yielded a visible cell suspension. DNA for PCR was obtained by boiling suspensions of purified *Achromatium* cells in TE buffer. 16S rRNA gene fragments were amplified using primers pA (5′-AGAGTTTGATCCTGGCTCAG-3′) (Edwards *et al.* 1989) and AO703r (5′-CTGGTATTCCTCCTGATATC-3′). AO703r was designed using the probe and PCR primer design software tool Primrose (Ashelford *et al.* 2002) and checked for specificity using the Ribosomal Database Project (RDP II) probe match analysis tool (Cole *et al.* 2007). Comparison of the primer sequences with RDP II Release 9.47 (Feb 1, 2007) identified a number of matches with non-target sequences. However, AO703r is selective for *Achromatium* 16S rRNA gene sequences and the use of purified *Achromatium* cell suspensions, ensured that only 16S rRNA gene fragments from *Achromatium* spp. were amplified using pA and

AO703r. This was tested empirically, by excising and sequencing bands from DGGE gels and analysis of 16S rRNA gene clone libraries obtained with pA and AO703r.

PCR amplification was carried out according to a previously described method (Rowan *et al.* 2003) with an annealing temperature of 62^{o}C. PCR products were obtained from all 30 experimental microcosms except for a single replicate of the 90% Rydal Water: 10% Hell Kettles mix. PCR products generated using pA and AO703r were amplified using Primer 3 (5′-CCTACGGGAGGCAGCAG-3′) containing a GC clamp (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGG CACGGGGGG-3′) and Primer 2 (5′-ATTACCGCGGCTGCTGG-3′) (Muyzer *et al.* 1993). PCR products were purified using a Qiagen PCR clean up kit (Qiagen, Crawley, UK). DGGE analysis was conducted using the D-Gene denaturing gradient gel electrophoresis system (Bio-Rad, Hercules, CA, USA). Polyacrylamide gels (10 % polyacrylamide; 0.75 mm thick; 16 by 16 cm) were run in 1 X TAE buffer (40 mM Tris-acetate, 1 mM EDTA pH 8.3). A denaturant gradient ranging from 30-52% denaturant (100% denaturant is 7 M urea plus 40 % vol/vol formamide in 1 X TAE) was used. Gels were run at 60°C for 4 hours at a constant 200 V and stained for 30 minutes in SYBR green I (Sigma, Poole, UK; diluted 1/10000 in 1 X TAE). Stained gels were viewed using a Fluor-S MultiImager (Bio-Rad, Hercules, CA, USA).

Validation of the PCR-DGGE approach.

To validate the PCR-DGGE approach used to study *Achromatium* communities, bands present in DGGE profiles were confirmed as being derived from *Achromatium* spp. by comparative analysis of partial 16S rRNA gene sequences. Sequences were obtained either directly from bands excised from DGGE gels and/or on the basis of co-migration of bands in DGGE profiles with cloned *Achromatium*

16S rRNA gene fragments. Bands were excised from the DGGE gel using a sterile plastic pipette tip. DNA was eluted from the gel fragments by incubation in 50 μl of TE buffer (20 min, 20°C). An aliquot of the eluted liquid (1 μl) was used as template for amplification with primer 2 and primer 3 (Muyzer *et al.* 1993). The 16S rRNA fragments (each approximately 190 bp in length) were sequenced using Primer 2. A clone library of longer 16S rRNA gene fragments was generated from purified *Achromatium* cells obtained from Rydal Water sediment using primers pA and AO703r. PCR-amplified 16S rRNA gene fragments were purified using a Qiagen PCR clean up kit (Qiagen, Crawley, UK) and cloned using a Qiagen PCR cloning kit (Qiagen, Crawley, UK) with the pDrive cloning vector and Qiagen EZ competent *E. coli*. All procedures were carried out according to the supplier's instructions. White colonies containing inserts were selected at random and DNA was extracted by boiling cells picked from the colony in TE Buffer for 3 minutes. Amplification of DNA inserts was performed using primers pUCr (5'-CAGGAAACAGCTATGAC-3') and pUCf (5'-GTTTTCCCAGTCACGAC-3') according to a previously described method (Rowan *et al.* 2003). PCR products of the correct size from 50 randomly selected clones were screened by DGGE, after amplification with primers 2 and 3 (see above) to identify groups of different sequences from the clone library.

Eight groups of clones were identified by their migration in DGGE gels. Representative clones from each of these groups were sequenced. Initially 23 of the cloned 16S rRNA genes were sequenced. This demonstrated that each of the groups identified on the basis of their migration in DGGE gels was homogenous. On this basis the sequence of the entire amplified 16S rRNA gene fragment (ca. 700 bp.) was determined for a representative clone from each of the groups, using the primer pC (5'-CTACGGGAGGCAGCAGTGGG-3') (Edwards *et al.* 1989). These eight 16S

rRNA sequences have been deposited in the GenBank database with accession numbers (DQ288131-DQ288138). All sequencing was conducted using the DyeDeoxy chain termination method using a 3730 xl DNA analyzer (Applied Biosystems).

Phylogenetic distance analysis was conducted using the Jukes and Cantor (Jukes and Cantor 1969) correction for multiple substitutions at a single site and the neighbour joining method (Saitou and Nei 1987) as implemented in the TREECON package (van De Peer and De Wachter 1994). Bootstrap re-sampling was conducted with 100 replicates. The analysis included 34 sequences and covered positions 100-202 and 221-722 (*E. coli* numbering). Comparison of the short sequences obtained from DGGE bands with the GenBank database using BLAST (Altschul *et al.*1990), and with sequences obtained in this study confirmed that all sequences represented *Achromatium* spp.

For analysis of *Achromatium* community DGGE profiles from the microcosms, the Bionumerics software package (Applied Maths, Austin, Texas, US) was used to produce a normalized composite gel with reference to markers included on the DGGE gels (van Verseveld and Röling 2004). Band matching data were exported to a Microsoft Excel spreadsheet for subsequent analysis. Primer 6 for Windows (Version 6.1.5, Primer-E Ltd., UK) was used to construct Bray-Curtis similarity matrices for non-metric multidimensional scaling (MDS) followed by Analysis of Similarities (ANOSIM) using band designation as variables of presence and absence (Clarke and Warrick 2001).

The MDS algorithm uses distance information from a Bray-Curtis similarity matrix to place each data point in low dimensional space whilst maintaining distance relationships between each point. The resulting MDS plots are representations of how

different the treatments are from each other based on clustering of like samples. To determine if the observed clusters are statistically significant, a pairwise analysis of similarity (ANOSIM) was conducted on the similarity matrix. ANOSIM generates an R statistic, which is calculated by comparing the mean distances within user-defined groups and between groups (the latter of which should be greater if the groups are different), and ranges from 0 (completely random pattern) to 1 (treatments are completely separated). A p-value is then computed by randomizing the samples to determine if the R value generated from the user-defined groups is greater than any randomly generated R value.

Results

Diversity of Achromatium in the Rydal Water and Hell Kettles sites and validation of the PCR-DGGE approach employed.

A total of 6 bands was excised from a DGGE profile (Fig. 1) from a microcosm which comprised 75% Rydal Water and 25% Hell Kettles sediments (time zero, replicate 2). Four band derived fragments (designated C1, C2, C3 and C6) that were also present in the parent Rydal Water community were sequenced. In addition two further sequences (designated as C4 and C5) were obtained which corresponded with the two principle bands present in the Hell Kettles parent community. Comparative sequence analysis demonstrated that all of the six sequences from the excised DGGE bands where related to *Achromatium* sequences previously recovered from the Rydal Water and Hell Kettle sites (Gray et al., 1999a).

As an additional confirmation of the specificity of the PCR-DGGE approach comparative analysis of the 50 cloned 16S rRNA gene fragments selectively amplified from purified Rydal Water *Achromatium* cells demonstrated that all eight

sequence types identified (designated RYA to RYH) clustered with the *Achromatium* assemblage within the *Gammaproteobacteria* (Fig. 2). Of these RYA, RYB, RYD, RYE and RYH all co-migrated with bands identified in DGGE profiles from the microcosms (Fig. 1).

Achromatium community size in the experimental microcosms

Differences in total *Achromatium* community size in the experimentally mixed microcosms (Fig. 3) were as a consequence of differences in the *Achromatium* community size in the parent sediments from which they were constructed. Hell Kettles sediment sustained approximately 10 times more *Achromatium* cells than Rydal Water sediment. Accordingly the microcosms represented a gradient of increasing *Achromatium* community size correlated with increasing contributions from the Hell Kettles sediment. On this basis, although the ratio of the two sediments (Rydal Water : Hell Kettles) by volume in the microcosms were 100:0, 90:10, 75:25, 50:50 and 0:100, the estimated ratios of cells and hence specific *Achromatium* sustaining resources contributed by the parent sediments (Rydal Water %±SE: Hell Kettles %±SE) were 100:0, 41±25:59±25, 21±15:79±15, 8±6:92±6 and 0:100. In the sediment microcosms incubated for 60 days, all replicate microcosms still contained *Achromatium* cells (Fig 3). For all but one of the experiments (100% HK microcosms) no significant difference ($p > 0.05$) in the total abundance of the *Achromatium* cells was found between day 0 and day 60. Replicating cells were observed both at time zero and after 60 days in all of the microcosms, however, despite the relatively steady state population sizes, cell turnover rates could not be calculated because *Achromatium* spp. have not been cultured in the laboratory and no realistic estimate of division times are available.

Achromatium community composition in the experimental microcosms

A composite gel including DGGE profiles from all of the microcosms (Fig 4a.) was subjected to numerical and statistical analysis (Fig 4b). It should be noted that the profiles are derived from purified preparations of *Achromatium* cells. It is unlikely, therefore, that any bands were derived from extra-cellular DNA released from dead cells.

Comparison of *Achromatium* community structure by MDS analysis (Fig. 4b) showed that at time zero *Achromatium* communities from the parent sediments 100% RY (filled red diamonds) and 100% HK (dark blue filled inverted triangles) fell into two mutually exclusive clusters which where separated along the abscissa. This indicated that the *Achromatium* community structure in the two *Achromatium* parent communities was different at the outset of the experiment, a finding which was consistent with previous studies of Rydal Water and Hell Kettles which demonstrated that the different sediments were dominated by distinct *Achromatium* lineages (Gray *et al.* 1999a). After 60 days the DGGE profiles from the unmixed sediments, 100% RY (open red diamonds) and 100% HK (open dark blue triangles) were still separated on the abscissa to the same extent. A comparison of the two groups by ANOSIM confirmed the separation of these communities with the maximum R statistic of 1 ($p < 0.01$). Thus during the incubation period both the *Achromatium* population size (Fig. 3) and community composition (Fig. 4), were broadly maintained in the parent sediments.

Analysis of the DGGE profiles from microcosms containing mixtures of sediments, at the start of the experiment, indicated that a gradient of diversity had been established based on the initial sediment ratios. At time zero (filled symbols in

3b) the 75% RY:25% HK microcosms (filled green squares) grouped mid way along the abscissa but were not significantly separated from the time zero microcosms containing 90% RY, 10% HK sediment (filled magenta circles) or microcosms comprising 100% Rydal Water sediment (filled red diamonds) ($R = -0.108$, $p = 0.29$; ANOSIM). They were, however, separated from the communities in time zero microcosms containing 100% HK sediments (filled dark blue inverted triangles), and with one exception the communities from microcosms containing 50% RY and 50% HK sediment (filled light blue triangles) ($R = 0.723$, $p = 0.036$; ANOSIM). This gradient of diversity, graphically represented by variation along the abscissa of the MDS plot is consistent with the ratio of sediment from each source in the microcosms and the observed differences in population size (Fig. 3). For instance in the time zero microcosms containing 90% RY, 10% HK sediment the estimated contribution of RY cells determined by cell counts was 41%. This correlates well with the relative intensity of bands attributable to RY *Achromatium* (39.7 ± 15.2) in the corresponding DGGE profiles. In the 75:25 sediment mix the estimated contribution of Rydal cells determined by cell counts was 21% and the relative intensity of bands attributable to RY *Achromatium* was 26.7 ± 7.3%. Importantly, these results indicate that there is little bias in the PCR amplification of 16S rRNA genes from the different *Achromatium* communities when mixed together.

After 60 days incubation (open symbols in Fig. 4b) there was no longer any significant difference ($R = -0.105$, $p = 0.64$; ANOSIM) between the *Achromatium* communities in the microcosms containing 75% RY sediment and 25% HK sediment (open green squares) and the *Achromatium* communities in the sediment containing 100% HK sediment (open blue inverted triangles) and the communities in microcosms containing 50% RY and 50% HK sediments (open light blue triangles)

profiles. However, there was now a significant difference between the communities in microcosms with 75% RY and 25% HK sediment (open green squares), the communities in microcosms with 90% RY and 10% HK sediments (open magenta circles) and the communities present in 100% RY sediment (open red diamonds) ($R = 0.728$, $p = 0.012$; ANOSIM). Taken togethert these data indicate a shift in the communities from mixed sediments, towards the composition of the Hell Kettle community and away from the Rydal community. After 60 days the relative intensity of bands attributable to Rydal Water *Achromatium* was reduced to 6.7 ± 3.1%. In contrast after 60 days the *Achromatium* communities in the microcosms with 90% RY and 10% HK sediment (open magenta circles) were still distinct from the *Achromatium* communities in microcosms containing 100% HK sediment (open inverted blue triangles) and those containing 50% RY and 50% HK sediment (open light blue triangles) ($R = 0.743$, $p = 0.005$; ANOSIM). In the MDS plots, the *Achromatium* communities in some of the microcosms were also clearly separated along the ordinate based on incubation time (ANOSIM ($R = 1$, $p < 0.001$).

Discussion

Validation of the experimental approach

It has been shown in experimental microcosms similar to those used here that redox gradients and the small-scale distribution of *Achromatium* species are rapidly re-established when microcosms are re-constituted from bulk sediments (Head *et al.* 2000). Furthermore, *Achromatium* community composition is maintained when re-constituted sediments are incubated (as in this study) with oxic overlying water (Gray *et al.* 2004). The microcosms used in this study therefore, are suitable vehicles for the experimental manipulation and perturbation of *Achromatium* communities.

Nevertheless, two important objectives of our experimental design were to validate the PCR-DGGE approach used for community analysis and to discriminate general experimental effects from putative environmental selection. To this end 'time zero' mixed sediment microcosms were sacrificed immediately to show which Rydal and Hell Kettles *Achromatium* species were detectable by the PCR-DGGE approach and, therefore, provide a reference pattern to identify experimental changes in the mixed sediments over time. The subsequent disappearance of RY bands after 60 days under certain environmental conditions therefore cannot be attributed to PCR bias since it is known that when RY cells are present alongside HK cells they were detectable in the DGGE profiles. In addition control microcosms containing sediment from a single source were prepared using the same procedures as microcosms containing mixtures of sediments from two locations. During the 60 day incubation, the *Achromatium* communities in these control microcosms did not separate along the abscissa in MDS plots as was observed in some of the mixed microcosms. In contrast significant separation of Achromatium communities along the ordinate of the MDS plots was observed in all control microcosms and in all microcosms containing sediment

mixtures. On this basis we conclude that distributions along the ordinate, are attributable to general time related effects of incubation in microcosms, whereas those along the abscissa can be attributed to environmental and community mixing and environmental selection (see below).

If environmental selection plays a pivotal role in determining the geographical distribution of related but genetically distinct species, it was reasoned that the selective pressure on species from different locations would correlate with the sediment they occupy. If sediments from two environments containing different *Achromatium* species are mixed, there will be a specific ratio of the two sediments where the species from one geographical location will be favoured over the other, due to the sediment conditions more closely resembling their home environment. This environmental selection and phenotypic differentiation would be manifest by the growth or maintenance over time, of particular *Achromatium* spp. accompanied by antagonistic effects on other *Achromatium* spp.

The role of environmental selection in the geographical distribution of Achromatium *spp.*

In the mixed microcosm experiments population size was not increased above the notional carrying capacity of the sediments even though *Achromatium* diversity was artificially increased. On this basis we did not anticipate, or observe (Fig. 3), a major decline in total *Achromatium* numbers in the mixed sediments during the course of the 60 day incubation. This precludes the exclusion of *Achromatium* sp. which were present and detectable in low abundance at time zero, simply on the basis of a general decline in the *Achromatium* population. On this basis the pattern of community change observed in microcosms containing 75% RY and 25% HK

sediment i.e. selection against the Rydal Water community and persistence of Hell Kettles species even when the sediment mix contained a substantial proportion of Rydal Water sediment, demonstrates that the Hell Kettles *Achromatium* species have a selective advantage in the mixed sediments. This selective advantage was not found to be significant as the sediment mixture became dominated by Rydal Water sediment (90% RY, 10% HK, Fig. 4b). It should be noted (see above) that the notional resources contributed to the microcosms based on the numbers of cells sustained in the unmixed sediments meant that even the most Rydal-like sediment mix (90% RY, 10% HK) had a dominance of Hell Kettles-derived resources at least in terms of those specific components of the resource pool which sustain *Achromatium* spp. if not the overall physico-chemical conditions.

Although we have not directly measured dispersal, our experiments represent an extreme manifestation of dispersal of *Achromatium* spp. from one location to another. Our results show, that even when there is large-scale immigration, the *Achromatium* community composition correlates with the nature of the host sediment. This indicates that environmental selection determines the composition of the *Achromatium* community in the two lakes. This experimental finding is consistent with observed environmental differences between the sites (Head *et al.* 2000) and some limited information on functional differences between the geographically separated *Achromatium* populations. Rydal Water has low sulfate (15-168 nmol cm^{-3}) and high organic carbon (14.8-15.9 wt %) concentrations, Measured sulfate reduction rates are in the range of 10-300 nmol cm^{-3} d^{-1} and reduced sulfur species concentrations range from 3.4-9.5 μmol cm^{-3}. Hell Kettles sediments typically contain higher concentrations of sulfate (9870-11160 nmol cm^{-3}) and organic carbon (21.3-22.2 wt%) which result in higher rates of sulfate reduction (1824 ± 73 nmol cm^{-3} d^{-1})

and higher concentrations of reduced sulfur species (16.01-95.32 μmol cm^{-3}).It has also been shown (by an inability to amplify specific gene targets) that unlike Rydal Water populations, Hell Kettles *Achromatium* may lack the enzymes APS reductase and ribulose bisphosphate carboxylase/oxygenase (RuBisCO) suggesting that the metabolic potential of the populations is different. Regardless of these functional differences, it has now been experimentally shown that the realisable ecological niches of the *Achromatium* species from Rydal Water are not present in the Hell Kettles sediment. Accordingly, regardless of the frequency of dispersal, the Rydal Water species are unlikely to become predominant members of the Hell Kettles community. We conclude that at a regional level, dispersal and environmental selection are important factors in determining the distribution of specific bacterial taxa and if this translates to larger geographical scales then the global inventory of *Achromatium* will be principally dictated and restricted by habitat heterogeneity.

The initial driver for experimentally testing factors that lead to observed biogeographical patterns in bacteria was the ambiguity inherent in interpretation of purely observational environmental, functional and phylogenetic data. The default interpretation of biogeographical patterns is often endemism. However, it is apparent that these observed patterns can be interpreted in a number of ways as discussed above. Suggestions of microbial endemism (Bell *et al* 2005, Cho and Tiedje 2000, Green *et al* 2004, Oda *et al.* 2003, Papke *et al.* 2003, Reche *et al.* 2005, van der Gast *et al.* 2005, Whitaker *et al.* 2003) arising from allopatric speciation, are thought to be the consequence of segregation of populations when rates of evolution at a single location exceed rates of dispersal even in the absence of obvious physical barriers. Accordingly, the particular genetic marker (and its rate of evolution) used to identify putative endemic populations will affect the conclusions drawn. In this case we have

used a relatively conserved genetic marker (16S rRNA sequences) and have demonstrated that environmental selection is significant in dictating the biogeographical patterns observed in *Achromatium*. We have also provided an experimental approach that can be used to test the generality of this phenomenon in other bacterial taxa. Hitherto this has been an important barrier to rigorous interpretation of biogeographical patterns in bacterial communities (Fenchel and Finlay. 2005). Nevertheless, it is likely that in different environments and for different bacteria the relative importance of environmental selection and geographic dispersal in dictating microbial community structure will vary.

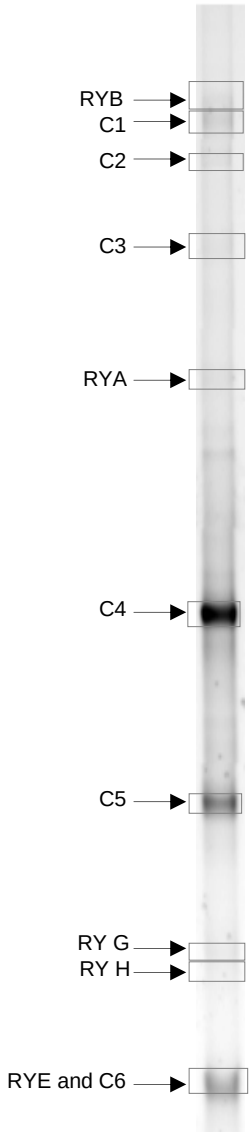

Figure 1. Band assignments for *Achromatium* 16S rRNA sequences obtained from bands excised from the DGGE gel (C1-C6) or by cloning and sequencing of a larger fragment of PCR-amplifed 16S rRNA gene (RYA, RYB, RYE, RYG and RYG).

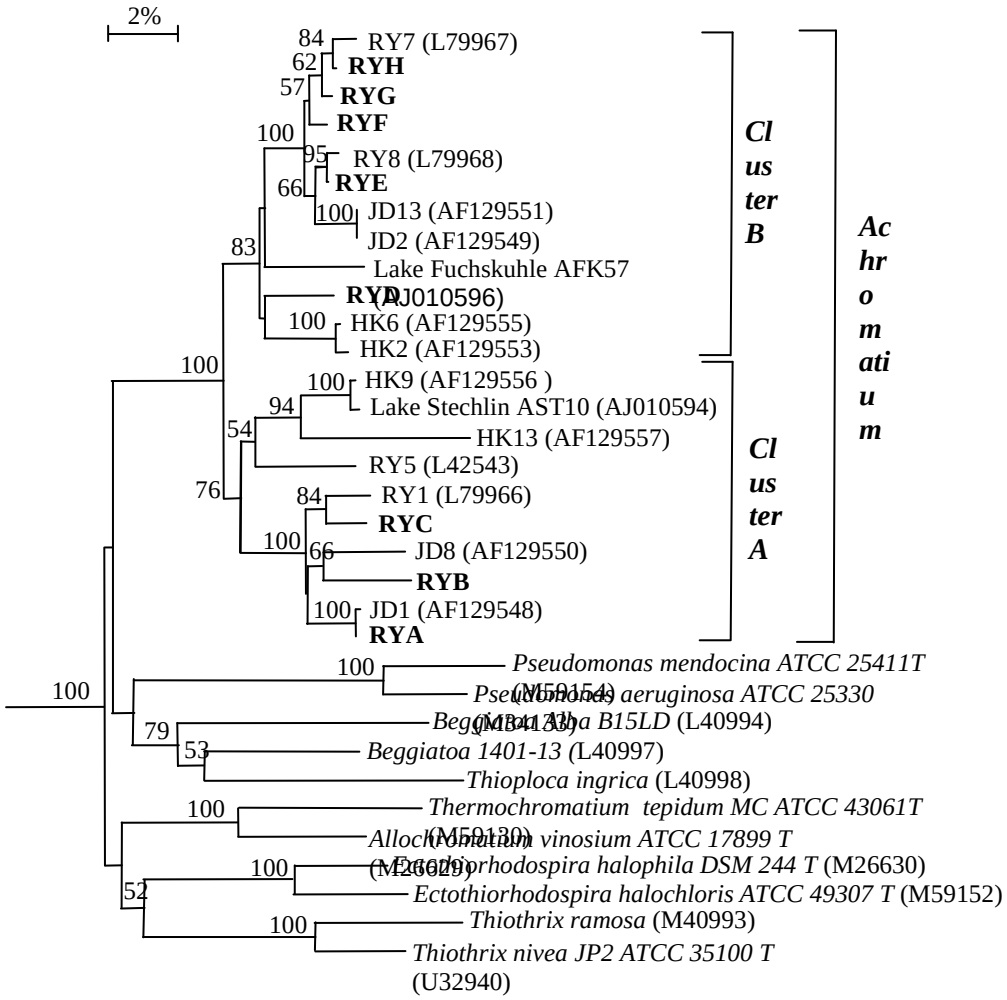

Figure 2. Phylogenetic distance tree based on the comparative analysis of partial 16S rRNA sequences (RYA to RYH) recovered from *Achromatium* cells purified from Rydal Water sediment. Fourteen *Achromatium* 16S rRNA sequences recovered from three sites in Northern England (Rydal Water (RY), Hell Kettles (HK), Jenny Dam (JD) and two sites in Germany (Lake Fuchskuhle, Lake Stechlin) (Glöckner *et al.* 1999) were included in the analysis. Genbank accession numbers for each sequence

are provided in parenthesis. The tree was rooted with respect to the *Desulfobacter postgatei* DSM 2034^{T} (M26633) 16S rRNA sequence. The scale bar denotes 2% sequence divergence and the values at the nodes indicate the percentage of bootstrap trees that contained the cluster to the right of the node. Bootstrap values less than 50 are not shown.

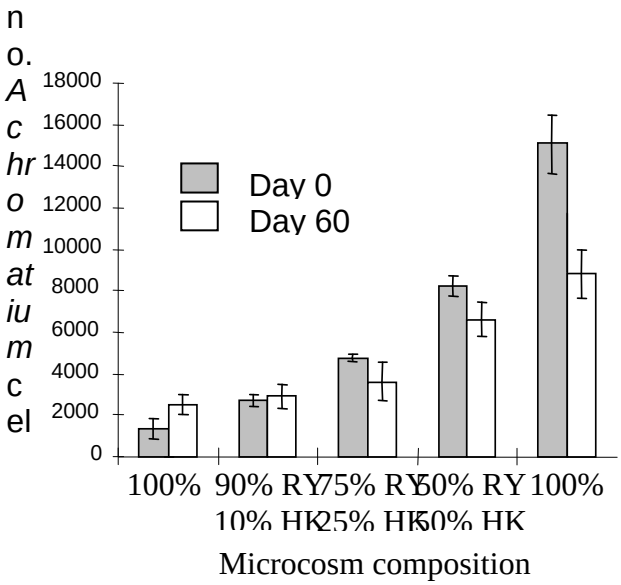

Figure 3. Abundanace of *Achromatium* cells in sediment microcosms. Microcosms at time zero (grey bars), microcosms after 60 days incubation (white bars). Error bars represent standard errors (n = 3). The sediment ratios (Rydal Water : Hell Kettles) by volume were 100:0, 90:10, 75:25, 50:50 and 0:100. The Hell Kettles sediment sustained approximately 10 times the number of *Achromatium* cells per unit volume than Rydal Water sediment. Accordingly, the estimated ratios of cells and resources contributed by the parent sediments (Rydal Water %±SE: Hell Kettles %±SE) were 100:0, 41±25:59±25, 21±15:79±15, 8±6:92±6 and 0:100.

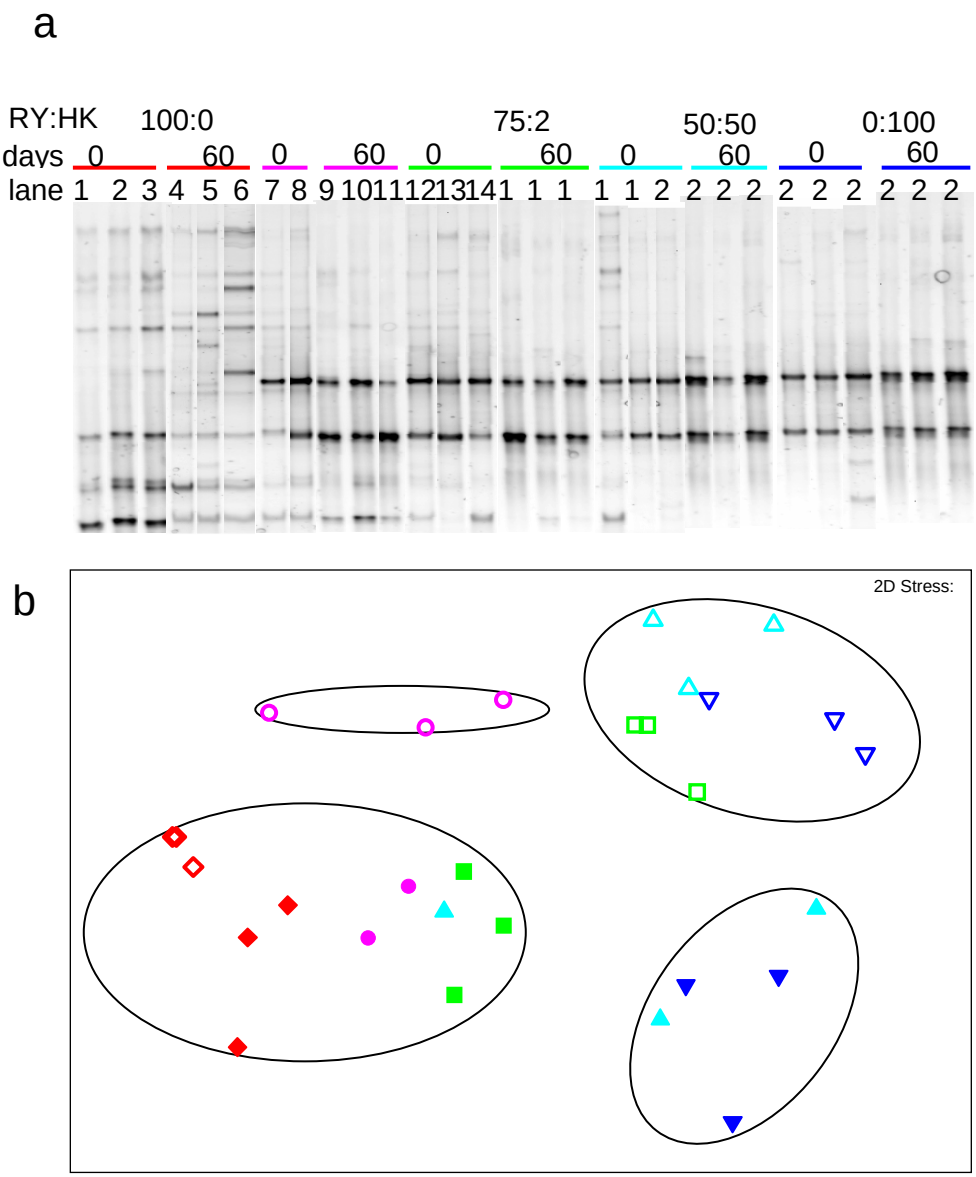

Figure 4. A. Composite DGGE gel of *Achromatium* 16S rRNA gene fragments derived from the experimental microcosms. Lane 1 to 3 and 4 to 6 represent, respectively, *Achromatium* community profiles from microcosms containing 100%

RY sediment at time zero (x3) and after 60 days (x3). Lanes 7 and 8 and 9 to 11 represent respectively, *Achromatium* community profiles from microcosms containing 90% RY and 10% HK sediment at time zero (x2) and after 60 days (x3). Lanes 12-14 and 15-17 represent respectively, *Achromatium* community profiles from microcosms containing 75% RY and 25% HK sediment at time zero (x3) and 60 day (x3). Lanes 18-20 and 21-23 represent respectively, *Achromatium* community profiles from microcosms containing 50% RY and 50% HK sediment at time zero (x3) and 60 day (x3). Lanes 24-26 and 27-29 represent respectively, *Achromatium* community profiles from microcosms containing 100% HK sediment at time zero (x3) and 60 day (x3).

B. MDS analysis ofDGGE profiles of *Achromatium* 16S rRNA gene fragments derived from the experimental microcosms. Coloured symbols indicate profile identity based on the ratio of sediment from the different lakes. RY: HK 100:0 (Red diamonds), 90:10 (magenta circles), 75:25 (green squares), 50:50 (light blue triangles), 0:100 (dark blue inverted triangles). The position of symbols reflects how different DGGE profiles are from each other based on their distance in a two dimensional plot. Distance is derived from Bray Curtis similarity coefficients calculated from the DGGE profiles. Filled symbols indicate replicate analyses from microcosms at time zero, open symbols indicate replicate analyses from microcosms following 60 days incubation.

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