Niche Partitioning Among Prochlorococcus Ecotypes Along Ocean-Scale Environmental Gradients

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Prochlorococcus is the numerically dominant phytoplankter in the oligotrophic oceans, accounting for up to half of the photosynthetic biomass and production in some regions. Here, we describe how the abundance of six known ecotypes, which have small subunit ribosomal RNA sequences that differ by less than 3%, changed along local and basin-wide environmental gradients in the Atlantic Ocean. Temperature was significantly correlated with shifts in ecotype abundance, and laboratory experiments confirmed different temperature optima and tolerance ranges for cultured strains. Light, nutrients, and competitor abundances also appeared to play a role in shaping different distributions.

The marine cyanobacterium genus Prochlorococcus (1) is the numerically dominant phytoplankter in tropical and subtropical oceans (2–5). It is distributed throughout the illuminated surface waters from about 40°N to 40°S, can be found as deep as 200 m, and sometimes reaches abundances greater than 10⁶ cells per ml (2). Prochlorococcus can account for 21 to 43% of the photosynthetic biomass in oligotrophic oceans and 13 to 48% of the net primary production (4–6). As such, its abundance and dynamics have a substantial impact on open ocean ecosystems and global biochemical cycles.

In the tropical and subtropical Atlantic Ocean, Prochlorococcus maintains its numerical dominance throughout most of the year, despite 10-fold changes in depth-integrated abundance (3, 6). It reaches its maximum (6, 7) when surface waters are highly stratified and devoid of major nutrients (8) and its minimum during deep winter mixing when blooms of Synechococcus and larger eukaryotic phytoplankton occur (3, 6, 7). Prochlorococcus abundance falls off sharply at higher latitudes, suggesting that temperature, or some related variable, is an important factor in shaping its global distributions (2).

Phylogenetic trees constructed with several marker genes, including small subunit (SSU) ribosomal RNA (rRNA), the rRNA internal transcribed spacer (ITS) regions, pshB, petB/D, and rpoC1, as well as whole-genome analyses, show that flow-cytometrically defined (9) Prochlorococcus is a genetically diverse group with several distinct clades, which have SSU rRNA gene sequences that differ by less than 3% (10–14). Cultured strains belonging to different clades have different pigmentation, maximum growth rates, metal tolerances, nutrient utilization, and physiotaxonomic characteristics, and thus these clades have been called ecotypes (15–17).

The ecotypes have recently been named after their photophysiological characteristics, and thus the ecotypes comprising this total had different adaptation was not the only variable shaping distributions. At 1°N, for example, eNATL2A and eMED4, low and high light-adapted clades, respectively, were roughly equal in abundance at the surface, whereas at 25°N eNATL2A was orders of magnitude less abundant than eMED4 near the surface. At high latitudes such as 48°N (Fig. 1B), one of the high light-adapted clades, eMED4, outnumbered the other, eMIT9312, by orders of magnitude in surface waters. Furthermore, the low light–adapted clade eMIT9313, which displayed deep subsurface maxima (~100 m) at low and midlatitudes, was so reduced in numbers at high latitudes that it was near the limit of detection. As has been previously reported (18, 19), two of the clades, eMIT9211 and eSS120, were present at low concentrations (~50 cells per ml) in all samples and thus were not included in this part of our analysis (fig. S4).

Similar shifts in ecotype dominance were also revealed by ecotype depth-integrated abundances along the entire transect (Fig. 1C), despite relatively constant (~1 × 10⁷ to 2 × 10⁷ cells per mm²) total Prochlorococcus concentrations. Overall, high light–adapted eMIT9312 was the dominant ecotype, exceeding the abundance of other ecotypes by two orders of magnitude between 15°N and 15°S. Low light–adapted ecotypes eNATL2A and eMIT9313 had roughly equal abundance in this part of the transect, and high light–adapted
eMED4 was the least abundant. By contrast, in cooler, well-mixed, nutrient-rich, high-latitude waters where eMED4 achieved numerical dominance, eMIT9313 disappeared, and eNATL2A achieved the second highest abundance. eMIT9312, the most abundant clade in the tropical and subtropical oceans, was below the detection limit at latitudes above 42°S. Our qPCR primers, designed from sequences of cultured isolates, captured on average ~75% (range from 35 to 118%) of the total Prochlorococcus population counted by using flow cytometry (Fig. 1C),
indicating that there are likely additional members not quantified in this study. However, there was no pattern to the residuals, suggesting that our observations were not greatly affected by culture bias (23).

Environmental factors and ecotype abundance. By using nitrate concentration to represent a suite of covarying nutrients (figs. S2 and S3), depth as related to light intensity, and plotting temperature contours on a slice through the upper 200 m along the transect, we visualized how ecotype abundances map on to environmental gradients (Fig. 1D). As expected, total Prochlorococcus cell concentrations were typically highest at or near the surface and decreased with depth. They were also highest in warm waters, which here are defined as waters above the 17°C isotherm, except below 150 m, where populations were likely limited by light availability. Lastly, total Prochlorococcus abundances were highest where nutrients were lowest, consistent with findings in other tropical and subtropical oligotrophic regions (4, 6, 7, 24).

These patterns of total Prochlorococcus abundance emerge from the distributions of ecotypes, which display more complex behavior when viewed individually. For example, the relative abundances of the high light–adapted ecotypes (eMIT9312 and eMED4) depended on latitude and closely followed nutrient and temperature contours (Fig. 1D). eMIT9312 dominated at low and midlatitudes but was progressively replaced by eMED4 at latitudes above 30°. Thus, these two ecotypes, which differ in their SSU RNA sequences by less than 1% (12), had overlapping but distinct niches in the Atlantic Ocean and comprised the majority (93%) of the Prochlorococcus cells over the transect.

Ecotypes eMIT9313 and eNATL2A had more restricted distributions. eNATL2A was most prevalent along the trailing edge of the eMIT9312 abundance contour and was most abundant in waters between the 15°C and 23°C isotherms. This distribution started at the surface waters of 45°N, meandered in the water column in mid- and low latitudes, and reached higher abundances again at the surface waters near 30°S. eNATL2A only reached high concentrations in deeper waters, where eMIT9312 and eMED4 numbers were reduced. Similarly, eMIT9313’s distribution was more patchy and localized to specific latitudes or depths, closely following the vertical gradients of eMIT9312 and eMED4, and was maximal where total Prochlorococcus abundance decreased the most sharply with depth. Thus, although eNATL2A and eMIT9313 were minor contributors to overall Prochlorococcus numbers in the Atlantic Ocean, they dominated at certain depths and latitudes.

Regression analysis. The geographic patterns of Prochlorococcus and its ecotypes suggest causal relationships with environmental variables (Fig. 1D). We used stepwise regression analyses to investigate relationships with temperature, light, nitrate, nitrite, ammonia, phosphate, and Synechococcus (a potential competitor) (table S1). As has been observed previously (25), a significant fraction (26%, P < 0.01) of the variability in the total Prochlorococcus population could be explained by temperature, which was largely driven by significant correlations between the numerically dominant ecotype, eMIT9312, and temperature (56%, P < 0.01). By using laboratory isolates belonging to the two dominant (and high light–adapted) ecotypes, we found that, although both had similar temperature optima for growth, members of eMIT9312 grew faster at this temperature (25°C) and could tolerate warmer temperatures than eMED4. The latter could grow at temperatures below 15°C, however, whereas eMIT9312 could not (Fig. 2 and fig. S5), thus confirming the importance of temperature in defining ecotype niche space.

Prochlorococcus ecotype abundances are related to other variables as well. For example, eMED4 was correlated (9%, P < 0.01) with light, which is consistent with its adaptations to high light (16). Nutrient relationships were complex (often being anticorrelated with ecotypes) (table S1) and were generally not consistent with the ability of strains to use nutrient sources (17, 26), suggesting that colinear variables not measured here were responsible for these relationships. Integrated abundances of Synechococcus (Fig. 1D), which unlike Prochlorococcus can use nitrate (17), were also inversely related to Prochlorococcus (fig. S6). This relationship has been found before for seasonal cycles in the Atlantic Ocean (3, 6) and implies that at high nutrient levels Synechococcus and larger phytoplankton may exclude Prochlorococcus. But this relationship with Synechococcus was complex: eMIT9313 was anticorrelated (22% of the variability), eMED4 (18%) and eMIT9312 (10%) were correlated, and the abundance of eNATL2A was uncorrelated.

The stepwise regressions, which had varying degrees of success in explaining the overall abundance patterns, also demonstrated that there were additional factors that were important in determining Prochlorococcus ecotype abundance. This complexity is likely governed by differential susceptibility to viruses (27) and protozoan grazers (28) as well as other factors, such as metal tolerances (15).

The relationships we have established here represent only a broad-brush assessment, to be refined as we learn more about Prochlorococcus microdiversity. By design, multiple ribotypes (based on ITS sequences) are represented in the ecotype populations that are enumerated by our qPCR primers (18), and it is likely that each of these is a physiological and genomic variant. This prompts the question: If one designed primers to enumerate ribotypes within the ecotype clusters, what would their distributions look like along these gradients? Would they be more similar to each other than to those belonging to other clades? Furthermore, although the four dominant ecotypes account for the majority of the total Prochlorococcus population, our analyses (Fig. 1), as well as evidence from clone libraries (18) and Sargasso Sea shotgun sequencing (29), reveal the existence of additional genotypes that do not group with any of the six defined genetic clades and thus would not be captured by our approach. These additional clades likely account for the difference between the total Prochlorococcus population measured by flow cytometry and the summed clades measured by qPCR (Fig. 1C), particularly in the deeper waters (Fig. 1B).

References and Notes
30. The authors acknowledge C. Robinson and the captain and crew of Royal Research Ship James Clark Ross for our participation in the AMT cruise and D. Veneziano for guidance with the statistical analysis. This work was funded in part by the Gordon and Betty Moore Foundation, the Seaver Foundation, NSF Biological Oceanography Division (to S.W.C.), and U.S. Department of Energy (to S.W.C.), NOAA and the University of Tennessee (to Z.I.), and NSF and the University of Tennessee (to E.R.Z.). It was also supported by the UK Natural Environment Research Council through the AMT consortium (NER/O/S/2001/00680). This is School of Ocean and Earth Science and Technology contribution no. 6686 and AMT contribution no. 107.

Supporting Online Material
www.sciencemag.org/cgi/content/full/311/5768/1737/DC1
Materials and Methods
Figs. S1 to S6

27 July 2005; accepted 11 January 2006
10.1126/science.1118052
Supporting Online Material for

Niche Partitioning Among Prochlorococcus Ecotypes Along Ocean-Scale Environmental Gradients


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DOI: 10.1126/science.1118052

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Online Supplemental Materials:

Methods

Sampling
Samples were taken on a research cruise from the UK to the Falkland Islands in September – October 2003. This cruise, AMT13, was part of a larger Atlantic Meridional Transect project detailed elsewhere (S1). Along the meridional transect, continuous measurements of red fluorescence (an indicator of phytoplankton biomass) and temperature were made. Discrete water samples for other measurements at ~10 depths in the upper ~200m were made using a CTD rosette system twice daily with the distance between stations averaging ~375km.

Cell Abundances
Cell abundance samples were frozen with 0.125% glutaraldehyde at -80°C until analysis following (S2). Prochlorococcus and Synechococcus were enumerated flow cytometrically using a modified EPICS V (Coulter) flow cytometer following (S3, S4). Populations were distinguished by characteristic size (scatter) and pigment (fluorescence) properties using FlowJo analysis software (TreeStar) (S4).

Ecotype Abundances
Prochlorococcus ecotype abundances were estimated using a quantitative PCR approach (qPCR) detailed elsewhere (S5, S6). Briefly, 100ml of sample water is filtered through a 25mm 0.22µm polycarbonate filter using gentle vacuum (<10in. Hg), followed by 3ml of an EDTA preservation solution. Triplicate filters are frozen (-80°C) individually in beadbeater tubes until later analysis. Cell lysis buffer (650µl) is added to the filters
and shaken (without beads) in a beadbeater at 4800RPM for 2 minutes. Cell lysate (500 µl) is incubated at 95°C for 15 minutes and then frozen (-80°C) for later use. Six unique qPCR reactions are setup, one for each of the six known Prochlorococcus ecotypes. Environmental samples are run in parallel with a range of serially-diluted standards from cultured members of defined clades. Standard curves typically have an $r^2=0.99$ or better and our typical detection limit is ~1 cell/ml.

**Nutrients**

The macronutrients were analyzed using a Bran and Luebbe segmented flow colorimetric auto-analyser. Nitrate, nitrite, ammonium, silicate and phosphate were measured following previously described protocols (S7, S8). All sampling and analytical procedures adopted 'clean' procedures, all samples were analyzed within 3 hours of sampling and no samples were frozen or stored. Where nitrate, nitrite and phosphate concentrations were depleted to nanomolar concentrations, they were analyzed using colorimetric segmented flow techniques coupled with Liquid Waveguide capillary cells and photodiode detection (S9). For the majority of the cruise track the ammonium concentrations are also below the detection limit of standard colorimetric segmented-flow autoanalytical techniques, so, analysis at the ambient nanomolar concentrations was carried out using a gas diffusion technique where the ammonia crosses a Teflon membrane due to a pH differential and then is measured fluorimetrically (S8, S10).

**Growth Rates**

Temperature-dependent growth rates for different Prochlorococcus ecotypes were measured on a temperature gradient bar with cultured strains growing in a 14:10 light
dark cycle at $66 \pm 3 \mu\text{mol} \text{ Q m}^{-2} \text{ sec}^{-1}$ following previous experimental designs (S11, S12).

**Stepwise Linear Regressions**

Stepwise linear regressions were made using MATLAB v7.0 using data that was normalized to a mean of 0 and standard deviation of 1. Light and cell concentrations were log transformed before analyses. Log transformation of nutrient data did not significantly improve regression fits. Before analyses, input variables were gridded at 3 degree latitude and 20m resolution, which corresponds to the average resolution of sampling.

**Supplemental Material References**


Figure S1

Schematic of the phylogenetic relationship among different Prochlorococcus type strains (after Rocap et al. 2002)
Figure S2

Depth distribution of ammonium, nitrite, silicate and phosphate, overlaid with temperature contour lines, along the transect.
Figure S3

Relationship between nitrate concentration, and that of phosphate and silicate in upper 200m along the transect.
Figure S4

Depth distribution of eSS120 and eMIT9211 ecotypes overlaid with temperature contour lines, along the transect.
Growth rate as a function of temperature for cultured strains belonging to the (A) eMIT9312 (yellow) and (B) eMED4 (green) clades. The upper bounds of these individual curves were used to create the clade patterns in Fig 2.
Relationship between depth integrated (0-200m) Prochlorococcus and Synechococcus (measured by flow cytometry) for all stations along the transect.
Table S1

<table>
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<th>Variable</th>
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<th>eMED4</th>
<th>eNATL2A</th>
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</table>

Percent variability of Prochlorococcus ecotypes explained by variables in a stepwise regression analyses. Only values significantly different (p<0.01) from zero are shown. Values in parentheses were anti-correlated.