Response to Luo & Konstantinidis: Phosphorus-related genes are enriched in Prochlorococcus populations from the North Atlantic

LETTER

Reply to Luo and Konstantinidis: Phosphorus-related genes are enriched in *Prochlorococcus* populations from the North Atlantic

Luo and Konstantinidis (1) assert that there is no difference in phosphorus (P)-related gene content between surface *Prochlorococcus* populations in the North Atlantic [Bermuda Atlantic Time Series (BATS)] and North Pacific [Hawaii Ocean Time Series (HOT)] and that mixing of ecotypes explains P-gene enrichment at BATS (2). We disagree.

P-Related Genes Are More Abundant at All Depths at BATS

At 20/25m, nine genes are significantly enriched at BATS relative to HOT, including alkaline phosphatase (*phoA*; Table 1); *phoBR* are also enriched although not statistically significant. At 50/75m —dominated by the high-light adapted ecotype eMIT9312 (Fig. 1) —22 genes are enriched at BATS, including *phoBR*, *phoA*, and the regulator *ptrA*. Although phosphonate utilization genes only appear in deeper waters (1), 19 other genes with experimental or bioinformatic evidence linking them to P limitation are enriched in the shallower depths at BATS (3) (Table 1). Thus, every depth contributes to the signal we observed (2). Furthermore, our results concur with another study (4) that examined *Prochlorococcus* P-related genes in surface waters only.

Ecotypic Structure Cannot Explain Differential P Gene Abundance

Although ecotype structure is not identical at a given depth between the two sites (Fig. 1 *A* and *B*), it is nearly identical when summed over the water column (Table S2 in ref. 2; Fig. 1*C*). Additionally, our sample collection at BATS preceded winter mixing (Fig. 1*D*); thus, advection of ecotypes (1) cannot easily explain our results. Further, if the latter were true, we would expect to find other ecotype-specific genes enriched at BATS, for instance low-light adaptive genes. More importantly, P-acquisition genes are decoupled from the core genome (ecotype) phylogeny (2–5) in *Prochlorococcus*; there is no evidence to date that particular ecotypes are adapted to low P.

P-Related Genes Originate from *Prochlorococcus*

The vast majority (83%) of shotgun clones we claim to carry a *Prochlorococcus* P-related gene match *Prochlorococcus* on both ends (Table 1; contrast with Table 1 from ref. 1, which shows only a fraction of the clones). Some do not (1, 2) (Table 1), but this is expected because P-related genes are known to occur in hypervariable islands in *Prochlorococcus* (2, 3). Further, known *Prochlorococcus phoBR* sequences cluster phylogenetically—and we have yet to find a *Prochlorococcus*-like *phoBR* sequence residing in a noncyanobacterial genome—suggesting that similar sequences from wild cells also likely derived from *Prochlorococcus* regardless of the adjacent genes. Last, an across-the-board 80% identity cutoff for calling a gene *Prochlorococcus*-derived is overly rigid, because we know, from sequenced genomes, that some genes are less conserved than others: for example, a P-regulated conserved hypothetical gene (P9301_12451), unique to *Prochlorococcus*, is only 72% identical between *Prochlorococcus* genomes.

Summary

Our conclusion that selection for P-related genes accounts for their enrichment in the North Atlantic is the most parsimonious explanation for our observations (2) and is consistent with previous studies (3, 4). Moreover, the overrepresentation of P-related genes in *Pelagibacter* at BATS (2) reinforces the significance of P-limitation as a selective agent. Given the complexity of natural systems, metagenomics will invariably present ambiguities; interpreting them can be informed by the experimental and genomic context provided by model organisms like *Prochlorococcus*.

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Table 1. *Prochlorococcus* genes that are differentially abundant between BATS and HOT at each depth

Locus	Gene	Predicted function	Linked to P-limitation?	Fraction of clones w/ *Prochl.* on both ends	HOT 25m		HOT 75m		HOT 110m		BATS 20m		BATS 50m		BATS 100m	
					Reads	Per cell	Reads	Per cell	Reads	Per cell	Reads	Per cell	Reads	Per cell	Reads	Per cell
P9301_12431	*phoB*	Phosphate response regulator	y (F, DE, N)	15/17	17	0.30	7*	0.09*	1*	0.04*	8	1.01	30*	0.83*	39*	1.09*
P9301_12411	*phoR*	Phosphate sensor kinase	y (F, DE, N)	25/28	12	0.13	12*	0.10*	0*	0.00*	9	0.72	50*	0.87*	50*	0.88*
P9312_07651		lipoprotein	y (DE, N)	7/8	0	0.00	0*	0.00*	0	0.00	1	0.28	10*	0.62*	8	0.50
P9312_07661	*phoA*	Alkaline phosphatase	y (F, DE, N)	42/45	5*	0.03*	10*	0.04*	0*	0.00*	35*	1.42*	113*	1.00*	112*	1.00*
P9301_12381	*chrA*	Chromate transporter	y (N)	35/36	20	0.21	13*	0.10*	3*	0.07*	6	0.45	49*	0.81*	54*	0.90*
P9301_12581	*arsA*	Arsenite efflux pump	y (F, N)	7/19	1	0.01	1*	0.01*	1*	0.03*	5	0.46	26*	0.52*	51*	1.03*
PMED4_07931	*ptrA*	CRP family regulator	y (F, DE, N)	7/14	0	0.00	0*	0.00*	0	0.00	2	0.34	15*	0.57*	15	0.57
P9301_12441		Hypothetical	n	4/4	0*	0.00*	3*	0.17*	0	0.00	5	2.75	20*	2.41*	12	1.45
P9301_12451		Hypothetical	y (DE, N)	21/23	4	0.13	6*	0.14*	1	0.07	6	1.38	22*	1.11*	21	1.06
PMED4_01321		Hypothetical	y (N)	17/21	0*	0.00*	0*	0.00*	0*	0.00*	17*	1.67*	39*	0.84*	30*	0.65*
PMED4_03931		Type-1 copper domain	y (N)	10/11	0	0.00	1*	0.02*	1	0.07	3	0.68	19*	0.95*	3	0.15
PMED4_15661		Phosphatase/ phosphohexomutase	y (F, N)	18/22	1*	0.02*	6*	0.09*	12	0.51	9*	1.23*	25*	0.75*	28	0.84
PMED4_16031		Hypothetical	n	3/4	0*	0.00*	1*	0.05*	0	0.00	5*	2.30*	17*	1.72*	10	1.01
PMED4_16061		Hypothetical	y (DE, N)	7/7	0	0.00	0*	0.00*	0	0.00	4	1.60	10*	0.88*	5	0.44
PMED4_16111		Hypothetical	y (N)	10/11	0	0.00	0*	0.00*	0	0.00	4	0.72	16*	0.63*	8	0.32
PMED4_16151		Hypothetical	y (DE, N)	5/6	0	0.00	1*	0.04*	0	0.00	0	0.00	14*	1.10*	4	0.32
PMED4_16191		Hypothetical	y (DE, N)	13/16	6	0.23	9*	0.26*	1	0.08	6	1.64	22*	1.32*	5	0.30
PMED4_16201		α/β Hydrolase	y (DE, N)	9/12	0*	0.00*	0*	0.00*	0	0.00	5*	0.47*	14*	0.29*	1	0.02
PMED4_16211		Hypothetical	y (DE, N)	21/21	0*	0.00*	0*	0.00*	0	0.00	12*	0.53*	27*	0.26*	5	0.05
PMED4_16261		Hypothetical	y (N)	5/8	0*	0.00*	3*	0.14*	0	0.00	6*	2.60*	19*	1.81*	3	0.29
P9215_12751		Possible protease	y (N)	13/20	2	0.04	2*	0.03*	0	0.00	4	0.65	20*	0.72*	12	0.43
P9301_12831		Predicted metal-binding	n		18	0.44	10*	0.19*	0	0.00	2	0.36	23*	0.90*	6	0.24
NATL2_15731		Hypothetical	n		0	0.00	0	0.00	52*	0.64*	1	0.04	0	0.00	25*	0.22*
NATL2_20401		Hypothetical	n		0*	0.00*	0	0.00	7	0.25	5*	0.58*	1	0.03	4	0.10
P9313_11911		Conserved hypothetical	n		25	0.45	35*	0.48*	7	0.28	0	0.00	1*	0.03*	4	0.11
P9313_11921		Metallophosphoesterase	n		36	0.56	28*	0.33*	12	0.42	0	0.00	0*	0.00*	5	0.12
P9301_17901	*galM*	Galactose mutarotase	n		54	0.80	119*	1.35*	30	1.00	9	0.97	19*	0.45*	40	0.95

Raw number of 454 reads is shown, along with copies per cell calculated as in ref. 2. Genes associated with P-limitation by at least one of three criteria are shown with a "y" in column 4 along with the applicable criteria: F, predicted function; DE, differential expression in response to P starvation (from ref. 3); and N, gene neighborhood (i.e., colocated with *Prochlorococcus* P-regulated genes on paired ends of BATS shotgun clones). To test whether these genes likely derived from *Prochlorococcus* cells, we identified BATS shotgun clones carrying each gene and asked how many of the paired end sequences also matched *Prochlorococcus.* The vast majority of shotgun clones are *Prochlorococcus*-like on both ends, implying they likely came from *Prochlorococcus* cells.
*$P < 0.05$.

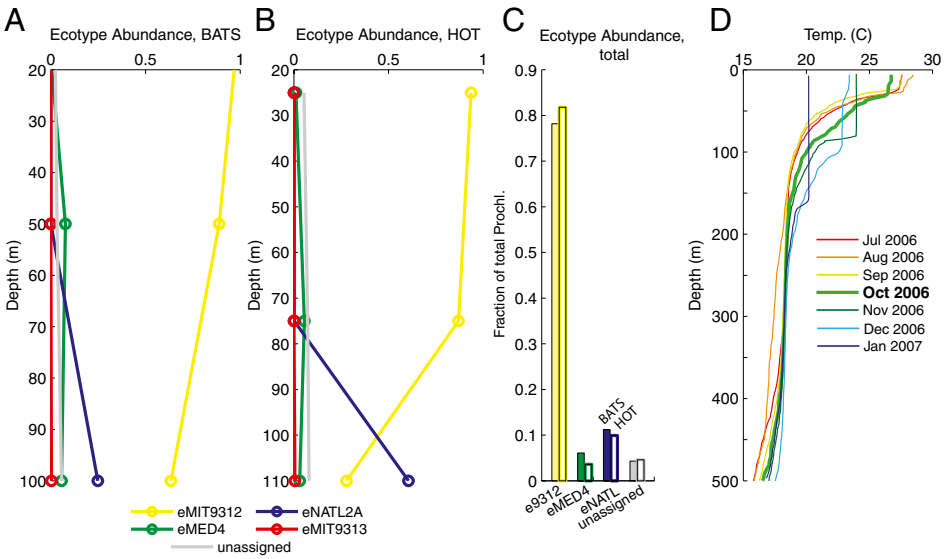

Fig. 1. Ecotype composition and mixing do not explain *Prochlorococcus* P-related gene enrichment at BATS. Ecotypes with depth (*A*) at BATS and (*B*) at HOT. (*C*) Summed ecotype composition over three depths. Ecotype abundance was determined by mapping 454 reads from core genes to sequenced genomes. Reads were only assigned to an ecotype if the top two genome hits belonged to the same ecotype. (*D*) Temperature profiles at BATS (data from http://bats.bios.edu), showing that the October 2006 profile, from which our samples were collected, looked more summer-like than winter-like, which does not support the ecotype advection hypothesis of Luo and Konstantinidis (1).