Report

Current Biology

Reacquisition of light-harvesting genes in a marine cyanobacterium confers a broader solar niche

Highlights

- Production of light-harvesting phycocyanin was lost in the *Acaryochloris* ancestor
- This trait was recently regained by an *Acaryochloris* strain and is highly plastic
- This confers a broader niche than that of relatives with the ancestral phenotype
- Bacteria can reacquire ancient lost traits by horizontal gene transfer

Authors

Nikea J. Ulrich, Hiroko Uchida, Yu Kanesaki, Euichi Hirose, Akio Murakami, Scott R. Miller

Correspondence

scott.miller@umontana.edu

In Brief

Most cyanobacteria primarily harvest light with phycobiliprotein complexes. Ulrich et al. show that the ability to produce these proteins was lost in the ancestor of *Acaryochloris* but has been recently regained by a member of this group via horizontal transfer. The loss of this complex trait was therefore reversible, even after millions of years.

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Report

Reacquisition of light-harvesting genes in a marine cyanobacterium confers a broader solar niche

Nikea J. Ulrich,[1] Hiroko Uchida,[2] Yu Kanesaki,[3] Euichi Hirose,[4] Akio Murakami,[2] and Scott R. Miller[1,5,*]
[1]Division of Biological Sciences, University of Montana, Missoula, MT, 59812, USA
[2]Kobe University Research Center for Inland Seas, Awaji, Hyogo, 656-2401, Japan
[3]Research Institute of Green Science and Technology, Shizuoka University, Shizuoka, 422-8529, Japan
[4]Department of Chemistry, Biology & Marine Science, Faculty of Science, University of the Ryukyus, Nishihara, Okinawa, 903-0213, Japan
[5]Lead contact
*Correspondence: scott.miller@umontana.edu
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SUMMARY

The evolution of phenotypic plasticity, i.e., the environmental induction of alternative phenotypes by the same genotype, can be an important mechanism of biological diversification.[1,2] For example, an evolved increase in plasticity may promote ecological niche expansion as well as the innovation of novel traits;[3] however, both the role of phenotypic plasticity in adaptive evolution and its underlying mechanisms are still poorly understood.[4,5] Here, we report that the Chlorophyll *d*-producing marine cyanobacterium *Acaryochloris marina* strain MBIC11017 has evolved greater photosynthetic plasticity by reacquiring light-harvesting genes via horizontal gene transfer. The genes, which had been lost by the *A. marina* ancestor, are involved in the production and degradation of the light-harvesting phycobiliprotein phycocyanin. *A. marina* MBIC11017 exhibits a high degree of wavelength-dependence in phycocyanin production, and this ability enables it to grow with yellow and green light wavelengths that are inaccessible to other *A. marina*. Consequently, this strain has a broader solar niche than its close relatives. We discuss the role of horizontal gene transfer for regaining a lost phenotype in light of Dollo's Law[6] that the loss of a complex trait is irreversible.

RESULTS AND DISCUSSION

A. marina MBIC11017 has re-acquired light-harvesting phycocyanin genes

The ability of organisms to respond to environmental change is integral to survival. The expression of alternative environmentally induced phenotypes from a single genotype, or phenotypic plasticity, can enhance fitness across a broader range of conditions than is possible for a single phenotype, both within an individual's lifetime and between generations.[2,7] However, the role of plasticity for adaptation has long been debated[5,8,9] and remains poorly understood for most organisms.[4,5] For instance, phenotypic plasticity itself might evolve, a process known as genetic accommodation, to obtain a better phenotype-environment match.[10,11] This could happen when a trait either loses (genetic assimilation) or gains environmental sensitivity.[1,2,12,13] On the other hand, it has also been argued that plasticity plays only a limited role in adaptation, for example, by acting as a buffer to environmental variation[1,5,14,15] or due to a lack of heritable variation for plasticity within populations.[9,16]

Addressing the role of phenotypic plasticity for adaptation therefore has implications for our understanding of the evolution of novel traits, the breadth of the ecological niche and the distribution of biological diversity.[17,18] In particular, the evolution of increased plasticity can promote diversification in spatially or temporally variable environments by facilitating the colonization of novel habitats or extending an organism's ecological range.[19–24] While a broader ecological niche tends to evolve in fluctuating environments,[25,26] it is still unclear how the mechanisms regulating plasticity and life history trade-offs interact to affect the evolutionary dynamics of populations.[5] A better understanding of the role of phenotypic plasticity for the evolution of ecological generalists and specialists is therefore required.

Here, we report that the evolution of increased plasticity by the reacquisition of lost genes has been central to niche expansion in the marine cyanobacterium *Acaryochloris marina*. This bacterium uniquely uses the far-red light absorbing Chlorophyll (Chl) *d* as its major photosynthetic pigment ([27]; >90% of chlorophyll content, compared with 1%–2% observed in some Chl *f*-producing bacteria). Like most cyanobacteria, the type strain *A. marina* MBIC11017[28] also produces bilin-containing phycobiliproteins (PBPs) to harvest light wavelengths that are not absorbed by chlorophyll pigments. Typically, cyanobacterial PBPs are organized in complexes called phycobilisomes (PBS), which are composed of a thylakoid membrane-associated core to which peripheral rods are attached.[29,30] PBPs absorb different wavelengths based on the structural variation of their attached bilin chromophores[31] and are arranged

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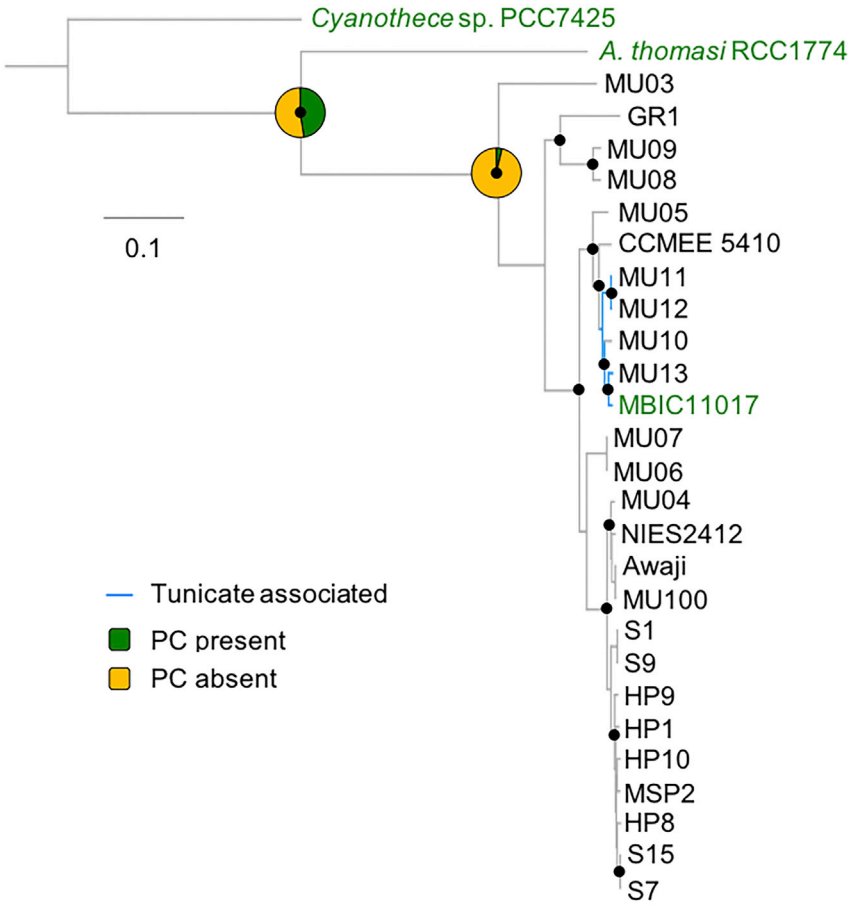

Figure 1. Reacquisition of phycocyanin production by *A. marina* MBIC11017

A maximum likelihood phylogeny of *Acaryochloris* strains was reconstructed from a genome-wide concatenation of protein sequences from single-copy orthologous genes (n = 1468). Branch lengths are in units of the expected number of amino acid substitutions per site. The tree was outgroup-rooted with *Cyanothece* sp. PCC7425, which harbors canonical PBS complexes and has been shown in previous phylogenies to be a member of the sister group to *Acaryochloris*.[40,41] Bootstrap support was >95% for each node. Internode certainty (IC) values (see STAR Methods) >0.2 are represented by closed circles. Bootstrap support for the split between *Acaryochloris* and RCC1774 was 100% with an IC value of 0.833. RCC1774 is also placed outside of the *A. marina* clade in a 16S rRNA gene phylogeny (Figure S2). Within the *Acaryochloris* clade, there is a general trend of increasing genome size from basal to more derived taxa (Table S2). Strains with phycocyanin are labeled in green. Pie charts display the marginal posterior probabilities of the presence or absence of phycocyanin in the common ancestors of *A. marina* and *A. marina*/*A. thomasi* RCC1774, respectively. The likelihood that the *A. marina* and RCC1774 common ancestor possessed PC was unresolved (52.8%), whereas the likelihood that the *A. marina* ancestor lacked PC was 96.3%. See also Figure S1, Figure S2, and Tables S1 and S2.

hierarchically within the PBS to promote the efficient transfer of excitation energy to the photosystem reaction centers.[30] However, *A. marina* MBIC11017 differs from other cyanobacteria in that its light-harvesting apparatus lacks an allophycocyanin (APC)-containing core and instead consists only of rods of the PBP C-phycocyanin (PC).[32–34] These rods are composed of heteromeric discs of α and β PC subunits as well as linker proteins that likely anchor PC to the thylakoid membrane and modulate the excitation energy transfer process.[35] Rods are arranged as stacks associated with the thylakoid membrane[34,36] and absorb primarily in the yellow-orange spectral range, with a peak at ~620 nm.

The origin of MBIC11017's novel PC light harvesting apparatus remains a puzzle. Since its isolation, a few other *A. marina* strains have been described,[27,37] all of which lack PC. By contrast, the recently described Chl b-producing (and Chl *d*-lacking) *Acaryochloris thomasi* RCC1774, which has been proposed to be a member of the genus based on a 16S rRNA gene phylogeny, also possesses PC aggregates rather than a conventional PBS.[38] This strain was also reported to produce small amounts of APC;[38] this seems unlikely, however, because, like MBIC11017, the RCC1774 genome does not contain *bona fide apc* genes but, rather, distant *apc* homologs (Figure S1).

To address the evolution, distribution and ecological consequences of this light-harvesting apparatus, we first sought to better resolve the *Acaryochloris* phylogeny with improved taxon sampling. We established a diverse collection of *Acaryochloris* strains isolated from various locations, including the west coast of the United States, the coast around Japan,[39] the Caribbean, and the Arabian Sea (Table S1). Using this collection, we next acquired Illumina sequence data and assembled draft genomes for 24 new strains (Table S2) for phylogenomic analysis.

We inferred a genome-wide amino acid phylogeny using a concatenation of protein sequences from single-copy orthologous genes (Figure 1). The tree was outgroup-rooted with *Cyanothece* sp. PCC7425, which has been shown to be a member of the sister group to *Acaryochloris*[40,41] and harbors canonical PBS complexes typical of most cyanobacteria. The phylogeny shows that MBIC11017 belongs to a recently derived clade of tunicate-associated *Acaryochloris* (Figure 1); specifically, both MBIC11017 and its closest relative, *A. marina* strain MU13 (which is identical to MBIC11017 in 16S rRNA gene sequence and differs genome-wide from MBIC11017 at only 1.7% of orthologous nucleotide positions), were isolated from species of the didemnid ascidian *Lissoclinum* (Table S1).

In contrast to Partensky et al.,[38] our phylogeny placed *A. thomasi* RCC1774 outside of the Chl *d*-producing *A. marina* clade with 100% bootstrap support (Figure 1). This result was also supported by trees reconstructed for individual protein-coding genes. The trees of individual genes used in a concatenated phylogeny can disagree with the concatenated tree and with each other for both technical reasons (e.g., insufficient data to

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resolve the relationships among taxa) and biological ones (e.g., incomplete lineage sorting, horizontal gene transfer). We used internode certainty (IC) values[42] to evaluate the agreement between individual gene trees with the topology of the concatenated phylogeny. The IC value is an entropy measure of the degree of conflict for an internal branch in a phylogeny due to discordance among trees in the concatenated alignment; a value of 1 indicates that all of the individual gene trees support the concatenated topology, whereas a value of 0 indicates equal support for a bipartition that conflicts with the concatenated tree. The IC value of 0.833 for the split between RCC1774 and the *A. marina* ancestor (Figure 1) indicates that nearly all of the individual trees (> 95%) support the topology of the concatenated phylogeny. Finally, a 16S rRNA gene phylogeny reconstructed by maximum likelihood also placed RCC1774 outside of the *A. marina* clade (Figure S2). The difference in topologies between our 16S rRNA gene tree and that of Partensky et al.[38] may be due to the improved sampling of *A. marina* diversity in our analysis.

Our expanded *A. marina* phylogeny enabled us to take an ancestral state reconstruction (ASR) approach to test whether the novel PC light harvesting apparatus of MBIC11017 was either (1) present in and vertically inherited from the *A. marina* ancestor or, alternatively, (2) absent in the *A. marina* ancestor and consequently more recently reacquired by HGT. Remarkably, MBIC11017 is the only strain within the clade of Chl *d*-producing *Acaryochloris* that possesses genes for and produces PC, and ASR strongly supports the hypothesis that the *A. marina* ancestor lacked PC (Figure 1; the marginal posterior probability that the ancestor lacked PC was 96.3%). We conclude that *A. marina* acquired PC genes by HGT.

In MBIC11017, all genes involved in PC synthesis and degradation are found on plasmid pREB3.[43] This includes second, divergent copies of the phycocyanobilin synthesis genes *hemO*, *hemH*, and *pycA*. Like other *Acaryochloris*, MBIC11017 has retained ancestral chromosomal copies of these genes (phycocyanobilin is also used as a chromophore in phytochrome proteins;[44]). By contrast, its sister taxon *A. marina* strain MU13 possesses about half of the pREB3 genes but lacks all genes required for the synthesis and degradation of the phycocyanin apoprotein as well as additional copies of phycocyanobilin synthesis genes (Figure 2). This indicates that PC-related genes on pREB3 were recently acquired via horizontal gene transfer (HGT) by an ancestor of MBIC11017 following the split with MU13. In addition, several of the *cpc* and *hem* genes have subsequently been duplicated since their acquisition (Figure 2).

HGT is well-documented in many cyanobacteria.[45–47] Genes involved in PBS formation, in particular, have a history of HGT, gene duplication and/or gene loss.[48–50] We used neighbor net analysis to attempt to identify the nature of the donor(s) of PBP genes to MBIC11017. Networks of individual phycocyanin genes were characterized by extensive loops (Figure 3, Figure S3), which corroborates that HGT of *cpc* genes has been pervasive. For many of these gene networks, MBIC11017 is not sister to either *Cyanothece* or RCC1774; rather, it often clusters among heterocyst forming cyanobacteria (Figure 3). Although the specific identity of the donor(s) is not clear, these results suggest that it may have been a member of the distantly related clade of heterocystous cyanobacteria. The novel PBP organization in MBIC11017 is therefore a product of its complex evolutionary history. Whether the common ancestor of *A. thomasi* RCC1774 and *A. marina* possessed PBPs is unresolved by ASR (Figure 1). However, the absence of both a conventional PBS and *apc* genes in RCC1774 (Figure S1) suggests that RCC1774 also regained PC by HGT. Given the topology of the networks, this likely involved a different donor(s) than in the case of MBIC11017 (Figure 3, Figure S3).

PBP gene acquisition leads to increased plasticity and a generalist phenotype in *A. marina* MBIC11017

Next, we investigated the consequences of PC reacquisition for physiology and fitness in different light environments. Many cyanobacteria are capable of chromatic acclimation, i.e., plasticity in the composition of light-harvesting pigments to tailor the photosynthetic apparatus to light quality.[51] MBIC11017 can alter the amount of PC relative to Chl *d* in response to changes in light quality ([52–54], Figure S4A) as a consequence of the differential expression of PC-related genes.[53,55] We predicted that MBIC11017 can chromatically acclimate to prevailing light availability better than other closely related *A. marina* strains and that this is beneficial in environments enriched with PC-absorbing light. The degree of plasticity in pigment composition was particularly strong at amber (600 nm peak), yellow (590 nm peak) and green (525 nm peak) wavelengths compared with the other light treatments (Figure 4A; Figure S4C). This resulted in strong PC-driven photosynthesis by MBIC11017 cells grown in these environments, as indicated by fluorescence excitation spectra, (i.e., action spectra for photosynthesis; Figure 4B). In contrast, we observed that *A. marina* strains MU13 and MU10 exhibited no plasticity in either pigmentation or light-harvesting ability in the different light environments (Figure 4B; Figure S4B).

To address the implications of PC production for the *A. marina* solar niche, we next assayed growth of MBIC11017 and MU13 in broad spectrum white light as well as far-red, amber, yellow and green light environments. Although both strains grew in white and far-red light environments, only MBIC11017 could grow in all of the environments tested (Figure 4C), including green wavelengths that are at the limits of PC absorption (Figure 4B). MU13, by contrast, could only grow in amber light (Figure 4C); growth in this environment appears to be supported by excitation of the Q_x absorption band of Chl *d* (Figure 4B; peak at 610 nm). Strikingly, a mere 10 nm shift to yellow light completely inhibited MU13 growth (Figure 4C). We conclude that MBIC11017 has a wider niche breadth than its close relative with a more specialized ancestral phenotype.

In most cases, horizontally acquired and duplicated genes are quickly purged from *Acaryochloris* genomes in the absence of selection to maintain them.[56] The retention of reacquired PC genes in the MBIC11017 genome therefore suggests that they have been selectively favored in the natural environment. MBIC11017 was isolated from the transparent ascidian *Lissoclinum patella*, which harbors the symbiotic cyanobacterium *Prochloron*.[28] Because *Prochloron* also lacks phycobiliproteins, PC-absorbing wavelengths remain available at low levels in this environment.[57] This differs from the filtered FR-enriched light of intertidal algae[57,58] and may have presented an ecological opportunity,[28,59] whereby a chance acquisition of additional light-harvesting proteins to utilize more varied light wavelengths might have been favored and retained. In addition, growth of MBIC11017 in white light has been previously linked to a suite

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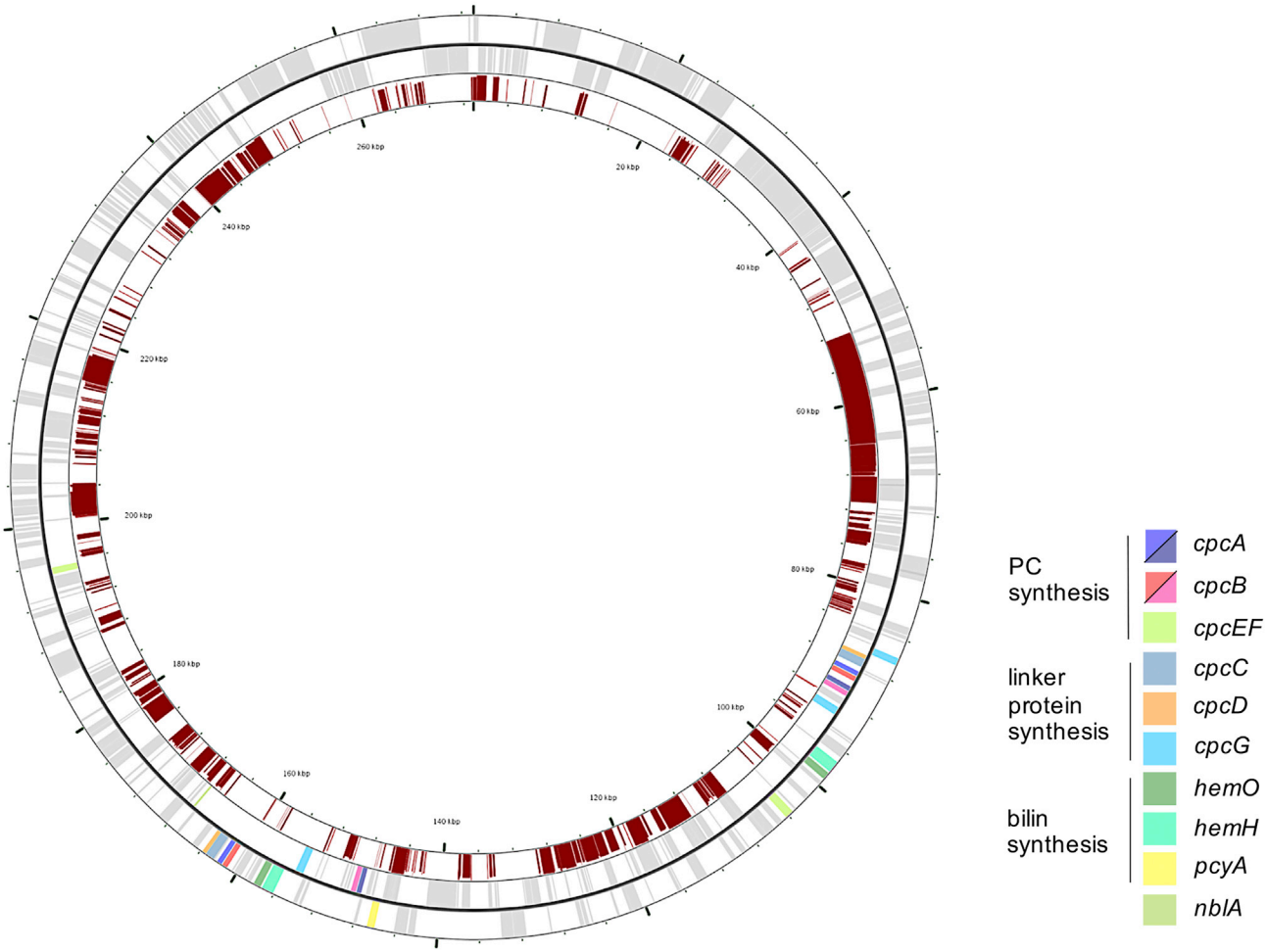

Figure 2. Distribution of phycocyanin-related genes on *A. marina* MBIC11017 plasmid pREB3

The different rings represent (from outer to inner): strand information for PBP-associated genes (rings 1 and 2, color-coded in key at right); and BLAST hits of the strain MU13 genome to pREB3 (ring 3, maroon). PC consists of a heterodimer of α and β peptide chains (encoded by *cpcAB*) that aggregate to form hexamers consisting of a trimer of dimers. Linker proteins encoded by *cpcCD* and *cpcG* anchor PC to the thylakoid membrane and modulate energy transfer to the photosystems.[29,35] Phycocyanobilin, the chromophore of PC, is an open chain tetrapyrrole synthesized from heme (produced by *hemH*) in a two-step process catalyzed by heme oxygenase (*hemO*) and phycocyanobilin:ferrodoxin oxidoreductase (*pcyA*). Phycocyanobilin is attached to PC cysteine residues via thioether linkages by phycocyanobilin α and β lyases (*cpcEF*), while *nblA* encodes the protein responsible for phycobiliprotein degradation.[29]

of traits associated with a planktonic lifestyle, including a reduction in biofilm formation.[53] We speculate that increased PC production could potentially enhance photosynthesis and fitness during planktonic dispersal, particularly at depths where far-red wavelengths cannot penetrate.

Our study also bears on the long-standing question in evolutionary biology of whether the loss of a complex trait (i.e., a trait composed of integrated parts, like a PC rod) is irreversible, as proposed in the modern formulation of Dollo's Law.[6] The law evokes well-known examples of apparently unidirectional evolution during biological diversification, such as the loss of teeth in birds. At the molecular and developmental levels, irreversibility is expected to arise as unused genes or regulatory networks accumulate inactivating mutations over time that make the reacquisition of a lost trait increasingly unlikely.[60] Proposed violations of the law remain rare and sometimes controversial, and the underlying molecular mechanisms responsible for the reacquisition of a trait are often poorly understood.[60] However, the latter is typically envisioned to involve the reactivation of genes that have been functionally retained in the genome due to their pleiotropic effects on other traits that have continued to be expressed. Though HGT is widely appreciated as a source of new traits, such as antibiotic resistance, we have shown that HGT also represents an alternative mechanism for bacteria to regain a trait via the acquisition of homologous genes, even after the ancestral copies have been lost. Our study is a striking example of how this was possible even following millions of years of *Acaryochloris* diversification.[37] Whether the reacquisition of ancient traits by HGT is common during bacterial diversification is unknown. We may expect this process to become more improbable with the increasing complexity of a trait's genetic architecture (e.g., the greater number of genes involved). On the other hand, the capacity for bacteria to organize functionally related genes as operons may facilitate HGT's role in re-evolving lost traits. Future comparisons of gene expression and

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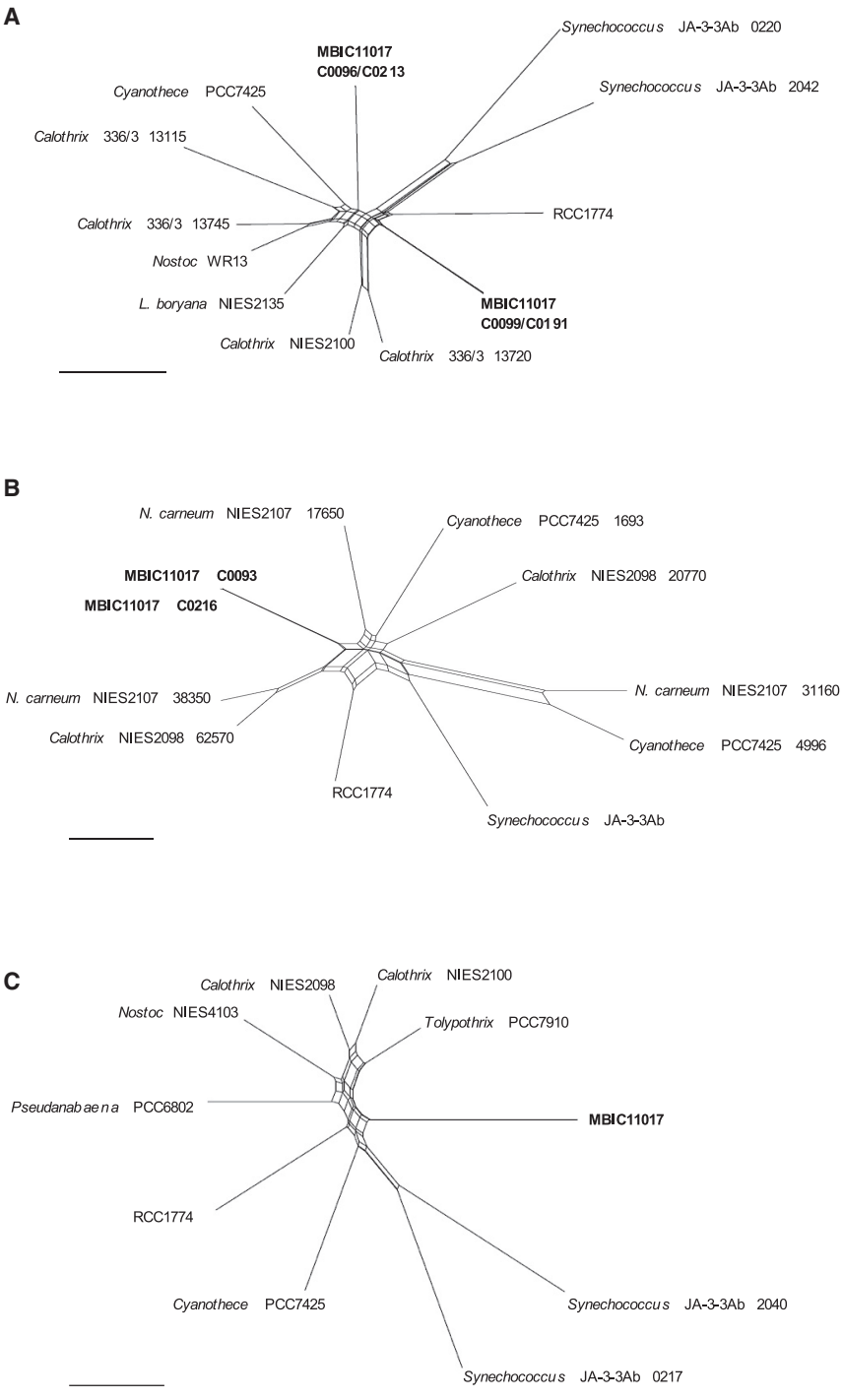

Figure 3. Neighbor net analyses of genes involved in phycocyanin synthesis

Networks are shown for (A) *cpcA*, (B) *cpcD*, and (C) *cpcE*. Taxa include those that have *cpc* genes most similar to *A. marina* MBIC11017 by NCBI BLAST as well as the outgroup *Synechococcus* JA-3-3Ab, which has been proposed to be a basal lineage in the cyanobacterial phylogeny.[37] Gene IDs are indicated for duplicated genes. The scale bars represent 0.1 nucleotide substitutions per site. See also Figure S3.

transcriptional regulation between MBIC11017 and MU13 will seek to reveal how these acquired genes were reintegrated into an existing transcriptional regulatory network that has evolved in their absence.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

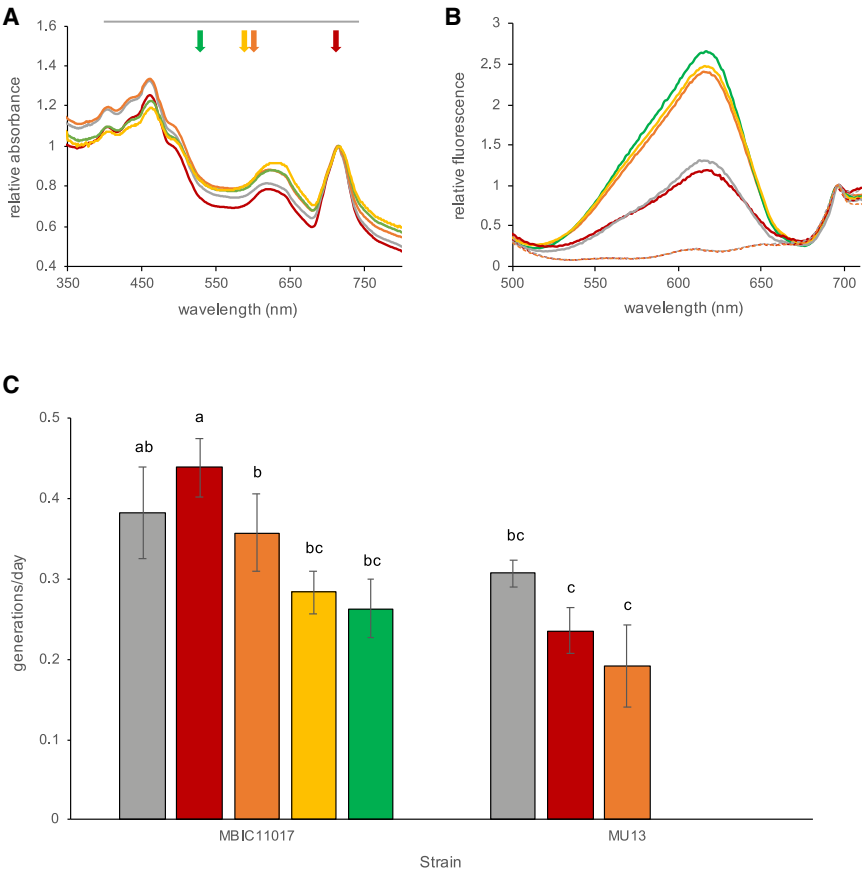

Figure 4. MBIC11017 and MU13 pigmentation, fluorescence and growth in different light environments

Data are color-coded by light environment in all panels.

(A) Absorption spectra for MBIC11017 normalized by the Chl *d* peak (696 nm). Colored arrows indicate the peak emission of the LED light environments: green (525 nm), yellow (590 nm), amber (600 nm), and far-red (710 nm); cool white fluorescent light emission is represented by the gray bar.

(B) Excitation spectra displaying relative fluorescence of MBIC11017 (solid lines) and MU13 (dotted lines) measured at an emission wavelength of 730 nm.

(C) Growth experiments of MBIC11017 and MU13 in far-red, cool white, amber, yellow, and green light. Different letters indicate a significant difference by a Tukey HSD post hoc test at the $p < 0.05$ level. Error bars are standard deviations for triplicate cultures. See also Figure S4.

- Phylogenetic analysis
- Growth experiments
- Chlorophyll d extraction and spectroscopy

● QUANTIFICATION AND STATISTICAL ANALYSIS

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

Conceptualization, N.J.U. and S.R.M; Methodology, N.J.U. and S.R.M; Investigation, N.J.U. and S.R.M.; Writing – Original Draft, N.J.U.; Writing – Review & Editing, S.R.M., N.J.U., H.U., Y.K., E.H., and A.M.; Funding Acquisition, S.R.M. and A.M.; Resources, S.R.M., H.U., Y.K., E.H., A.M.; Supervision, S.R.M.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
Acaryochloris marina S1	this paper	N/A
Acaryochloris marina S7	this paper	N/A
Acaryochloris marina S9	this paper	N/A
Acaryochloris marina S15	this paper	N/A
Acaryochloris marina HP1	this paper	N/A
Acaryochloris marina HP8	this paper	N/A
Acaryochloris marina HP9	this paper	N/A
Acaryochloris marina HP10	this paper	N/A
Acaryochloris marina MSP2	this paper	N/A
Acaryochloris marina GR1	this paper	N/A
Acaryochloris marina Awaji	this paper	N/A
Acaryochloris marina MU100	this paper	N/A
Acaryochloris marina MU03	this paper	N/A
Acaryochloris marina MU04	this paper	N/A
Acaryochloris marina MU05	this paper	N/A
Acaryochloris marina MU06	this paper	N/A
Acaryochloris marina MU07	this paper	N/A
Acaryochloris marina MU08	this paper	N/A
Acaryochloris marina MU09	this paper	N/A
Acaryochloris marina MU10	this paper	N/A
Acaryochloris marina MU11	this paper	N/A
Acaryochloris marina MU12	this paper	N/A
Acaryochloris marina MU13	this paper	N/A
Acaryochloris marina NIES2412	this paper	N/A
Acaryochloris marina MBIC11017	[43]	GCA_000018105.1
Acaryochloris marina CCMEE5410	NCBI	GCA_000238775.2
Acaryochloris thomasi RCC1774	[38]	NZ_PQWO00000000
Cyanothece sp. PCC7425	NCBI	NCBI:txid395961; GCA_000022045.1
Synechococcus sp. JA-3-3Ab	NCBI	NCBI:txid321327; GCA_000013205.1
Calothrix sp. 336/3	NCBI	NCBI:txid1337936; GCA_000734895.2
Calothrix sp. NIES-2100	NCBI	NCBI:txid1954172; GCA_002368195.1
Leptolyngbya boryana NIES-2135	NCBI	NCBI:txid1973484; GCA_002368255.1
Nostoc sp. WR13	NCBI	NCBI:txid2283151; MH918001

(*Continued on next page*)

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Nostoc carneum NIES-2107	NCBI	NCBI:txid1973483; GCA_002368155.1
Calothrix sp. NIES-2098	NCBI	NCBI:txid1954171; GCA_002368175.1
Tolypothrix sp. PCC7910	NCBI	NCBI:txid2099387; GCA_011769525.1
Pseudanabaena sp. PCC6802	NCBI	NCBI:txid118173; GCA_000332175.1
Nostoc sp. NIES-4103	NCBI	NCBI:txid2005458; GCA_002368335.1
Aulosira laxa NIES-50	NCBI	NCBI:txid1541988; GCA_002368055.1
Nostoc sp. CENA543	NCBI	NCBI:txid1869241; GCA_002896875.1
Tolypothrix tenuis PCC7101	NCBI	NCBI:txid231146; GCA_002368295.1
Nostoc sp. ATCC53789	NCBI	NCBI:txid76335; GCA_009873495.1
Trichodesmium erythraeum IMS101	NCBI	NCBI:txid203124; GCA_000014265.1
Calothrix brevissima NIES-22	NCBI	NCBI:txid1973478; GCA_002367995.1
Calothrix sp. NIES-3974	NCBI	NCBI:txid2005462; GCA_002368395.1
Mastigocoleus testarum BC008	NCBI	NCBI:txid371196; GCA_000472885.1
Chemicals, Peptides, and Recombinant Proteins		
Methanol	Roth	HN41.2; CAS: 67-56-1
Water	VWR Chemicals	23595.328; CAS: 7732-18-5
DNeasy PowerBiofilm DNA Extraction Kit	QIAGEN	CAS: 24000-50
Deposited Data		
Acaryochloris genome sequence data	This study	NCBI : PRJNA649288
Software and Algorithms		
Trimmomatic	[61]	V0.36
SPAdes	[62]	V3.12.0
QUAST	[63]	V4.5
Bandage	[64]	V0.8.1
CheckM	[65]	V1.0.18
BUSCO	[66]	V4.1.2
Rapid Annotation using Subsystem Technology (RAST)	[67]	V2.0
OrthoFinder	[68]	V2.2.7
IQ-TREE	[69]	V2.0
RAxML	[70]	V8.2.10
PastML	[71]	V1.9.29.9
SplitsTree	www.splitstree.org	V4.14.4
CGView	[72]	V1.0

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RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Scott Miller (scott.miller@umontana.edu).

Materials Availability

This study did not generate any new unique reagents.

Data and Code Availability

The accession number for the genome sequence data reported in this paper is NCBI: PRJNA649288.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Acaryochloris isolation and culture maintenance

Acaryochloris strains were isolated by incubating in far-red (FR) light (710 nm) in IO BG-11 medium, which consists of the following dissolved in 1 L of distilled water: 25 g Instant Ocean; 1.5 g $NaNO_3$; 0.0036 g $CaCl_2$-H_2O; 0.012 g $FeNH_4$Citrate; 0.001 g Na_2EDTA; 0.04 g K_2HPO_4; 0.075 g $MgSO_4$ x $7H_2O$; 0.02 g Na_2CO_3; and 1 mL of micronutrient solution (2.86 g H_3BO_3, 1.81 g $MnCl_2$ x 4H20, 0.222 $ZnSO_4$ x $7H_20$, 0.391 g Na_2Mo_4 x $2H_2O$, 0.079 g $CuSO_4$ x $5H_2O$, and 0.0494 g $Co(NO_3)_2$ x $6H_20$ dissolved in 1 L distilled H_20). IO BG-11 media was buffered with 10 mM HEPES (final) at pH 8.0.[37] Some strains were isolated in a modified saltwater medium (ASN-III) consisting of: 25 g NaCl; 3.5 g $MgSO_4$ x $7H_20$; 2.0 g $MgCl_2$ x $6H_20$; 0.75 g $NaNO_3$; 0.75 g K_2HPO_4 x $3H_2O$; 0.5 g $CaCl_2$ x $2H_2O$; 0.5 g KCl; 0.02 g $NaCO_3$; 3 mg citric acid; 3 mg ferric ammonium citrate; 0.5 mg EDTA; and 1 mL micronutrient solution (as above) dissolved in 1 L and adjusted to pH 7.4.[73] Batch cultures containing 75 mL of media were maintained in 250 mL Erlenmeyer flasks at 20°C or 30°C under 12 h cycles of 20-25 μmol photons m^{-2} s^{-1} of cool white fluorescent light. All strains have been deposited in the University of Montana Culture Collection for Cyanobacteria and are available upon request.

METHOD DETAILS

Genome sequencing, assembly, and annotation

Genomic DNA was extracted from *A. marina* strains using the DNeasy PowerBiofilm DNA extraction kit (QIAGEN) following the manufacturer's recommended protocol. Samples were sent to the University of Pittsburgh Microbial Genome Sequencing Center for library preparation and 150-bp paired-end sequencing on an Illumina NextSeq 550 platform. Sequence reads were trimmed of trailing low-quality bases and filtered based on read length and sequence quality with Trimmomatic version 0.36.[61] Draft genome assemblies for each strain were obtained with SPAdes version 3.12.0[62] using manually optimized parameter settings to maximize the N50. Assemblies were assessed with QUAST version 4.5,[63] and contaminant-derived contigs were removed by filtering by coverage with Bandage version 0.8.1.[64] Genome completeness was evaluated using CheckM version 1.0.18[65] and BUSCO version 4.1.2.[66] Assembled scaffolds for each strain were annotated using Rapid Annotation using Subsystem Technology (RAST).[67] Sequence data for all new strains can be found at NCBI BioProject PRJNA649288.

Phylogenetic analysis

Assembled genomes of 28 strains, including 24 obtained as part of this study and publicly available assemblies for *Acaryochloris marin*a MBIC11017 (NCBI accession GCA_000018105.1) and *Acaryochloris* sp. RCC1774 (NCBI accession NZ_PQWO00000000), were included in genome-wide phylogenetic reconstructions. We also used an improved assembly for *A. marina* strain CCMEE 5410 combining PacBio and Illumina data (NCBI BioProject ID PRJNA16707; 23 contigs, N50 = 4,516,345). These strains represent the known diversity of *Acaryochloris* and the outgroup *Cyanothece* sp. PCC7425 (NCBI accession GCA_000022045.1). A total of 1468 single copy groups of orthologous protein-coding genes were identified using OrthoFinder v2.2.7[68] to create a concatenated alignment of protein sequences. We constructed a maximum likelihood tree with 1,000 ultrafast bootstrap replicates[74] using IQ-TREE version 2.0[69] according to the JTT+R+F5 model of sequence evolution selected by the Akaike information criterion (AIC) in ModelFinder[75]. The Shimodaira-Hasegawa-like aLRT (SH-aLRT) was performed in parallel with bootstrap replicates to maximize load balance. Support for bipartitions in the concatenated tree was additionally provided by calculating internode certainty values.[42] To do so, individual maximum likelihood gene trees were inferred using IQ-TREE version 2.0 with 1,000 ultra-fast bootstrap replicates according to the model of sequence evolution selected with ModelFinder. Consensus trees for each individual protein-coding gene and the species tree were input to RAxML version 8.2.10[70] to calculate internode certainty and tree certainty values. In addition, a 16S rRNA gene phylogeny was constructed from nucleotide alignments using the above method according to the TIM3+F+R2 model of sequence evolution. Ancestral state reconstruction was performed with PastML[71] using the marginal posterior probabilities approximation (MPPA) maximum likelihood model and F81 model for character evolution.[76]

The genealogical relationships of individual *cpc* genes were inferred by neighbor net analysis from nucleotide alignments. Neighbor net analyses were implemented in SplitsTree version 4.14.4 (www.splitstree.org) for each PBP gene. Taxa with *cpc* genes with the

highest E values by NCBI BLAST to *A. marina* MBIC11017 were included. *Synechococcus* JA-3-3Ab was used as an outgroup because it is a basal lineage in the cyanobacterial phylogeny.[41] Accessions for additional sequence data used are as follows: GCA_000013205.1, GCA_000734895.2, GCA_002368195.1, GCA_002368255.1, MH918001, GCA_002368155.1, GCA_002368175.1, GCA_011769525.1, GCA_000332175.1, GCA_002368335.1, GCA_002368055.1, GCA_002896875.1, GCA_002368295.1, GCA_009873495.1, GCA_000014265.1, GCA_002367995.1, GCA_002368395.1, GCA_000472885.1. The circular genome map for *A. marina* MBIC11017 plasmid pREB3 was created by using the program CGView.[72]

Growth experiments

Growth was measured as the increase in culture optical density at 750 nm (OD_{750}) with a Beckman Coulter DU 530 spectrophotometer (Indianapolis, IN). For each strain, triplicate independent cultures derived from the same inoculum were grown in each light environment. LED boxes with peak emissions at 525 nm, 590 nm, and 600 nm, and 710 nm, respectively, were placed in 30°C incubators under continuous illumination. The LED lights varied marginally in light intensity: amber (4.0-4.96 μmol photons m^{-2} s^{-1}), yellow (3.4-4.0 μmol photons m^{-2} s^{-1}), green (5.5-6.04 μmol photons m^{-2} s^{-1}), white (7.5-8.1 μmol photons m^{-2} s^{-1}) and far-red (1.25-1.35 μmol photons m^{-2} s^{-1}). Growth was measured every 48 h by taking OD_{750} readings of a 2 mL subsample, after which cultures were randomly moved to different positions within each respective light environment to mitigate any differences in light exposure. Generation times were estimated from the exponential growth phase of each culture.

Chlorophyll d extraction and spectroscopy

Chl *d* concentration for each replicate was monitored by harvesting 2 mL of culture by centrifugation 14,000 X g for 5 min. Supernatant was discarded and cell pellets were resuspended in 2 mL ice cold 100% methanol by vortexing.[77] Samples were stored on ice in the dark for ~30 min to extract pigments, after which they were centrifuged at 14,000 X *g* to pellet cell debris. An absorbance scan (300-800 nm) was performed and Chl *d* peak was measured at 697 nm on a Beckman Coulter DU 530 spectrophotometer. The concentration of Chl *d* in μg/mL was determined using the published mass extinction coefficient of Chl *d* (63.68×10^3 L $mol^{-1}cm^{-1}$).[78] Aliquots of each culture were normalized by Chl *d* concentration before measuring excitation spectra (730 nm emission, 350-715 nm scan) with a Photon Technology International model QM-7/2005 spectrofluorometer (Ontario, Canada). Spectra were further normalized by peak wavelength.

QUANTIFICATION AND STATISTICAL ANALYSIS

Differences in growth rates of *Acaryochloris* strains MBIC11017 and MU13 in all light environments were assessed by Analysis of Variance (ANOVA) and Tukey HSD tests at a significance level of $\alpha = 0.05$ (Figure 4). Details of other quantification analyses can be found in the Results and Discussion text.

Current Biology, Volume *31*

Supplemental Information

Reacquisition of light-harvesting genes in a marine cyanobacterium confers a broader solar niche

Nikea J. Ulrich, Hiroko Uchida, Yu Kanesaki, Euichi Hirose, Akio Murakami, and Scott R. Miller

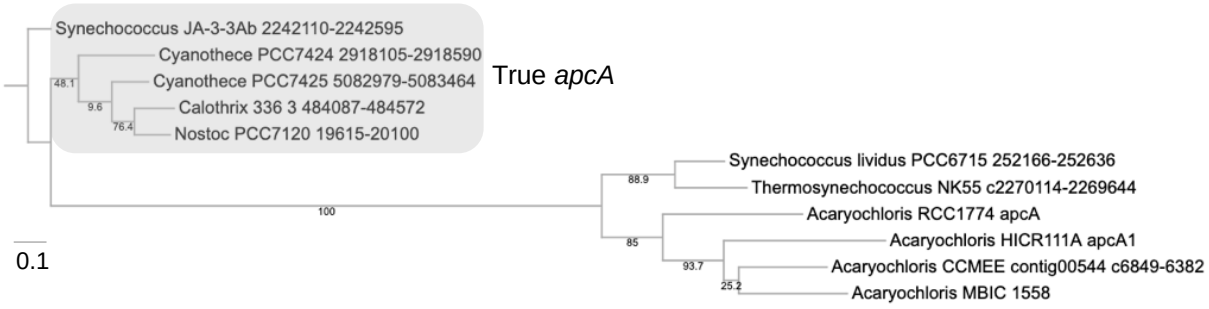

Figure S1. **Maximum likelihood gene tree of *apcA* and *apcA*-like homologs. Related to Figure 1.** The tree was reconstructed using the TIM3e+G4 model of sequence evolution. Bootstrap (n=1000) support is shown for each node. Taxa included are those that have genes most similar by BLAST to *A. marina* MBIC11017 as well as true *apcA* genes (shaded in gray). The scale bar is in units of expected number of nucleotide substitutions per nucleotide site.

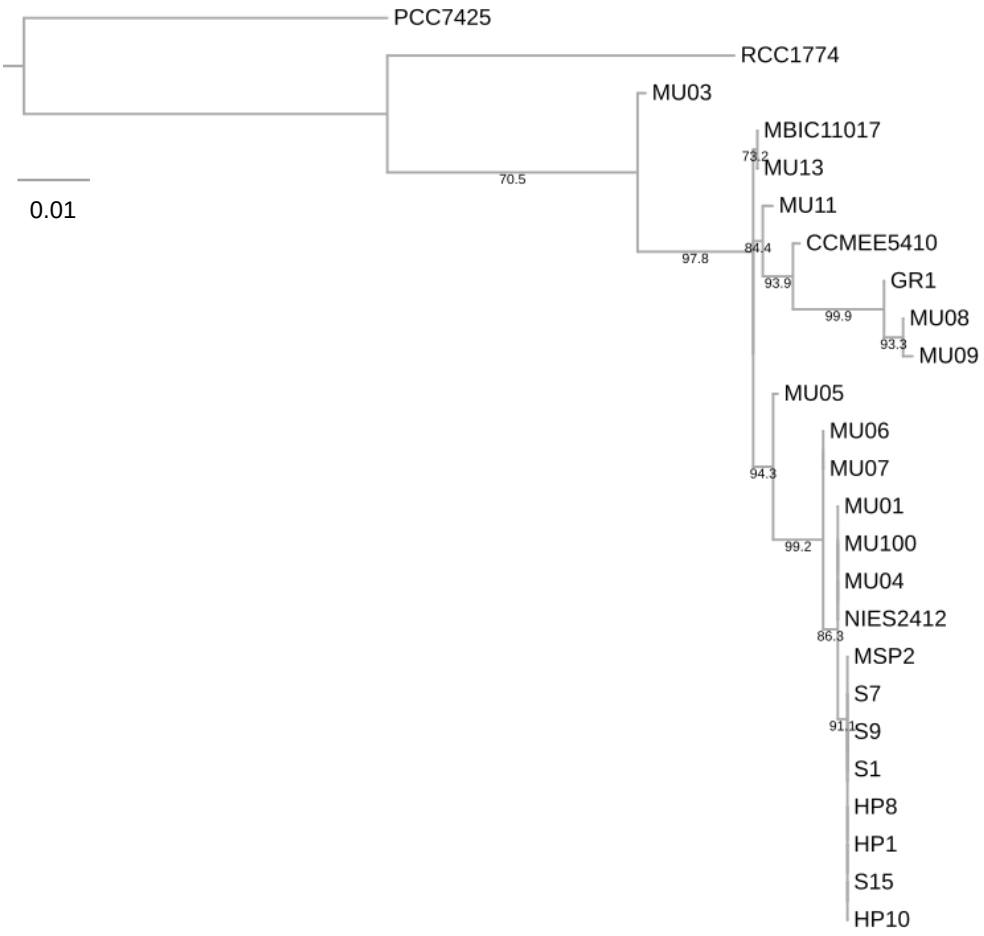

Figure S2. **16S rRNA gene phylogeny of *Acaryochloris* strains outgroup-rooted with *Cyanothece* sp. PCC7425. Related to Figure 1.** The tree was reconstructed by maximum likelihood according to a TIM3+F+R2 model of sequence evolution. Bootstrap (n=1000) support is shown for each node. The scale bar is in units of expected number of nucleotide substitutions per nucleotide site.

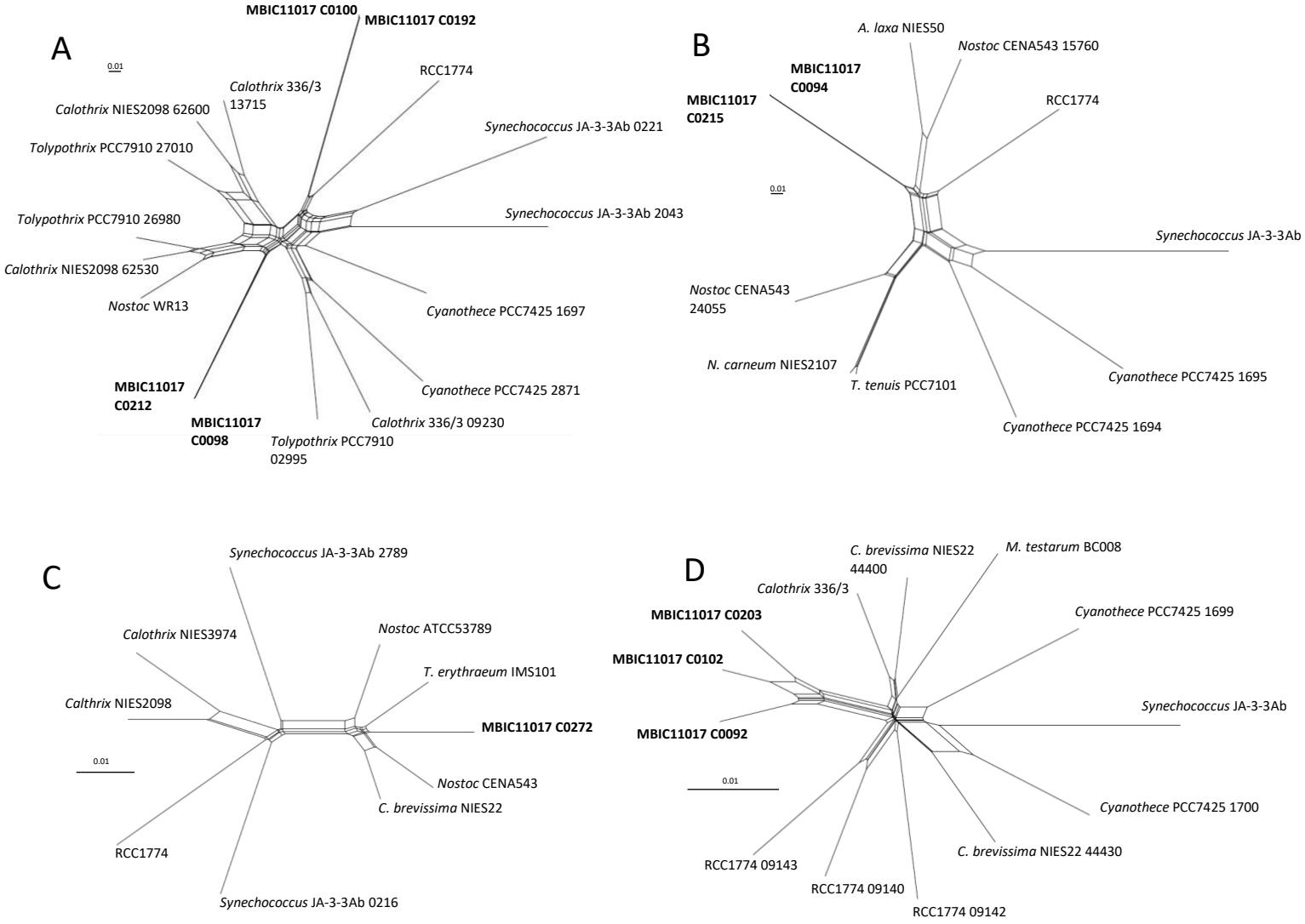

Figure S3. **Neighbor net analysis (NNA) of the relationships among cyanobacterial strains for additional PC-associated genes. Related to Figure 3.** Genes include *cpcB* **(A)**, *cpcC* **(B)**, *cpcF* **(C)**, *cpcG* **(D)**. Taxa included are those that have *cpc* genes most similar by BLAST to *A. marina* MBIC11017, as well as the outgroup strain *Synechococcus* JA-3-3Ab, which is a basal lineage in the cyanobacterial phylogeny [S1]. In the case of duplicates, gene ID is included in the taxon label. The scale bars are in units of substitutions per site.

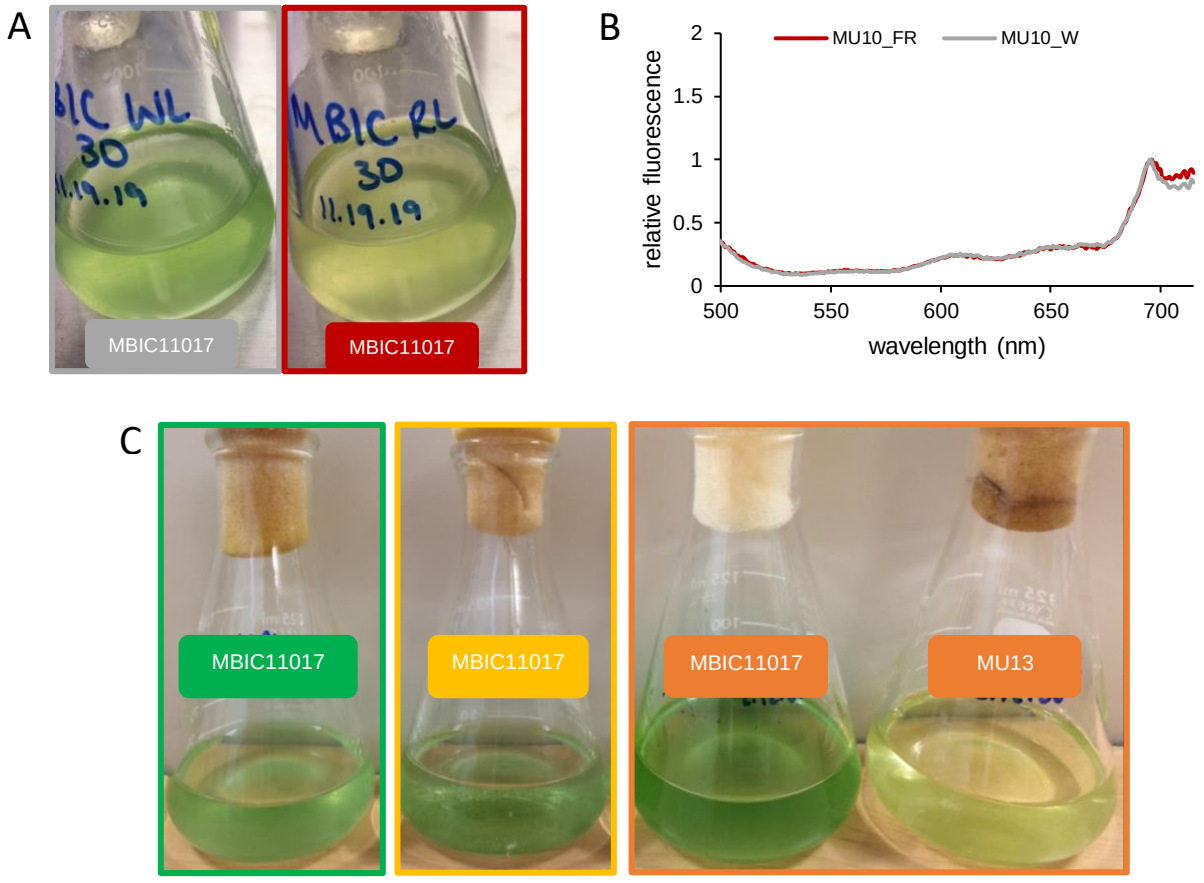

Figure S4. Pigmentation of *A. marina* MBIC11017 and closely related strains in different light environments. Related to Figure 4. MBIC11017 cells grown in white light (left) and far-red light (right) **(A)**. Excitation spectra displaying relative fluorescence of MU10 in far-red and white light **(B)**. Pigmentation of MBIC11017 and MU13 in different light environments **(C)**. From left: MBIC11017 in green (525 nm) light, MBIC11017 in yellow (590 nm) light, MBIC11017 in amber (600 nm) light, and MU13 in amber light.

Strain	Location	Sampling info	Collection Date
S1	Shelter Cove, CA	Upper intertidal pool, from surface of red algae (*Mastocarpus* sp.)	7/20/16
S7	Shelter Cove, CA	Surface of *Corallina* sp.	7/20/16
S9	Shelter Cove, CA	Surface of *Schizymenia pacifica*	7/20/16
S15	Shelter Cove, CA	Surface of *Pikea pinnata*	7/20/16
HP1	Hug Point State Park, OR	Algae on rocks at low tide	6/28/17
HP8	Hug Point State Park, OR	Algae on rocks at low tide	6/28/17
HP9	Hug Point State Park, OR	Algae on rocks at low tide	6/28/17
HP10	Hug Point State Park, OR	Algae on rocks at low tide	6/28/17
MSP2	MacKerricher State Park, CA	Algae on rocks or loose sand at low tide	5/28/18
GR1	Glover's Reef, Belize	Red algae from floating lagoon mat	1/16/18
Awaji (MU01)	Esaki, Awajishima, Hyogo, Japan	Epiphyte of red alga, *Ahnfeltiopsis flabelliformis*	2001
MU100	(derived from MU01)	Isolate as sterilized strain	3/27/15
MU03	Muroran, Hokkaido, Japan	Epiphyte of red alga, *Ahnfeltiopsis flabelliformis*	3/7/06
MU04	Muroran, Hokkaido, Japan	Endophyte of red alga, *Ahnfeltiopsis flabelliformis*	3/7/06
MU05	Arabian Sea	Epiphyte of red alga, *Gelidium* sp.	5/3/06
MU06	Tanoshiro, Awajishima, Hyogo, Japan	Epiphyte of red alga, *Callophyllis japonica*	6/9/06
MU07	Yura, Awajishima, Hyogo, Japan	Epiphyte of red alga, *Callophyllis japonica*	8/10/06
MU08	South China Sea	Epiphyte of red alga (unidentified)	1/8/07
MU09	Enoshima, Kanagawa, Japan	Epiphyte of red alga, *Ahnfeltiopsis flabelliformis*	2/3/07
MU10	Gushikami, Okinawa, Japan	Epiphyte of red alga (unidentified)	2/7/07
MU11	Bise, Okinawa, Japan	Isolate from tunicate, *Diplosoma virens*	4/3/07
MU12	Bise, Okinawa, Japan	Isolate from tunicate, *Trididemnum clinides*	4/3/07
MU13	Kurimajima, Okinawa, Japan	Isolate from tunicate, *Lissoclinum punctatum*	12/10/08
NIES2412	Muroran, Hokkaido, Japan	Seaweed (unidentified)	6/20/04

Table S1. New *Acaryochloris* strains included in this study. Related to Figure 1.

Strain [a]	N50	# contigs	Estimated genome size	Completeness (%) [b]	BUSCO (%)
S1	61925	398	7345427	99.29	98.9
S7	54529	446	7118253	99.53	99
S9	58396	410	7273775	99.29	98.9
S15	5881945	7	7112772	99.53	99
HP1	36836	890	7392918	99.29	98.9
HP8	39624	1098	7987243	99.29	99
HP9	36507	820	7003118	99.53	98.8
HP10	37829	1214	7998694	99.29	99
MSP2	48560	761	7090956	99.29	98.9
GR1	41795	470	6175695	99.53	97.8
Awaji (MU01)	17134	1153	7923976	98.47	95.2
MU100	48724	758	7664765	99.29	98.7
MU03	72346	458	6106866	99.53	98.8
MU04	43413	616	6967674	99.53	98.9
MU05	102057	138	5973023	96.7	96.5
MU06	27254	1219	8857603	99.76	98.7
MU07	32970	1060	8711329	99.76	99
MU08	35868	443	6609766	98.82	98.5
MU09	52586	337	6056097	98.58	98.3
MU10	64774	293	6553268	99.53	98.7
MU11	42252	406	7305935	99.53	98
MU12	16188	795	7405669	98.82	93.9
MU13	45821	486	7134557	99.53	98.6
NIES2412	41849	895	7860937	99.29	98.7
CCMEE5410	4516345	23	8072368	99.53	98.5
MBIC11017	6503724	10	8361599	99.53	98.9

Table S2. Genome assembly statistics. Related to Figure 1.

[a] MBIC11017 assembly is from Swingley et al. [S2]; all other assemblies are from this study
[b] Genome completeness was assessed using CheckM v1.0.18

Supplemental References

S1. Uyeda, J.C., Harmon, L.J., and Blank, C.E. (2016). A comprehensive study of cyanobacterial morphological and ecological evolutionary dynamics through deep geologic time. PLoS One *11*, e0162539.

S2. Swingley, W.D., Chen, M., Cheung, P.C., Conrad, A.L., Dejesa, L.C., Hao, J., Honchak, B.M., Karbach, L.E., Kurdoglu, A., Lahiri, S., *et al.* (2008). Niche adaptation and genome expansion in the chlorophyll *d*-producing cyanobacterium *Acaryochloris marina*. Proc. Natl. Acad. Sci. *105*, 2008.