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Dynamics of Gene Duplication in the Genomes of Chlorophyll d-Producing Cyanobacteria: Implications for the Ecological Niche

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Data deposition: This Whole Genome Shotgun project has been deposited at DNA Data Bank of Japan/EMBL/GenBank under the accession AFEJ00000000. The version described in this paper is the first version, AFEJ01000000

Abstract

Gene duplication may be an important mechanism for the evolution of new functions and for the adaptive modulation of gene expression via dosage effects. Here, we analyzed the fate of gene duplicates for two strains of a novel group of cyanobacteria (genus Acaryochloris) that produces the far-red light absorbing chlorophyll d as its main photosynthetic pigment. The genomes of both strains contain an unusually high number of gene duplicates for bacteria. As has been observed for eukaryotic genomes, we find that the demography of gene duplicates can be well modeled by a birth–death process. Most duplicated Acaryochloris genes are of comparatively recent origin, are strain-specific, and tend to be located on different genetic elements. Analyses of selection on duplicates of different divergence classes suggest that a minority of paralogs exhibit near neutral evolutionary dynamics immediately following duplication but that most duplicate pairs (including those which have been retained for long periods) are under strong purifying selection against amino acid change. The likelihood of duplicate retention varied among gene functional classes, and the pronounced differences between strains in the pool of retained recent duplicates likely reflects differences in the nutrient status and other characteristics of their respective environments. We conclude that most duplicates are quickly purged from Acaryochloris genomes and that those which are retained likely make important contributions to organism ecology by conferring fitness benefits via gene dosage effects. The mechanism of enhanced duplication may involve homologous recombination between genetic elements mediated by paralogous copies of recA.

Key words: Acaryochloris, recA, homologous recombination, plasmid.

Introduction

Gene duplication is an important mechanism of gene innovation and genome evolution (Ohno 1970; Taylor and Raes 2004). A substantial fraction of eukaryotic, bacterial, and archaeal genomes may be composed of divergent paralogs resulting from gene family expansion (Coissac et al. 1997; Jordan et al. 2001; Gevers et al. 2004; Makarova et al. 2005), and examples of the role of gene duplicates as a source of raw material for the origin of evolutionary novelties and diversification abound (e.g., True and Carroll 2002; Irish and Litt 2005; Wagner 2008).

In addition to ancient paralogs, eukaryotic genomes generally contain a large number of recent duplicates (Lynch and Conery 2000, 2003). By contrast, although gene duplications can occur at frequencies as high as $10^{-3}$ per gene per
Materials and Methods

Acaryochloris Strain CCMEE 5410 Genome

Cells were grown, and genomic DNA was isolated as previously described (Swingley et al. 2008). The CCMEE 5410 genome was sequenced on the 454 FLX Titanium platform and assembled with Roche's Newbler de novo assembler with default overlap settings. The JCVI auto-annotation pipeline was used to identify sequence features and assign functional annotation. Protein-coding sequences were predicted with Glimmer3 (Delcher et al. 1999), tRNAs were identified with the tRNAscan tool (Lowe and Eddy 1997), and rRNA genes and other structural RNAs were identified directly from Blast (Altschul et al. 1990) matches to Rfam. Functional annotation of proteins was assigned based on coding sequence comparison against the CHAR database of experimentally verified proteins and functional annotations, TIGRFAM (Haft et al. 2003) and Pfam (Finn et al. 2008) protein family databases, the PANDA repository of nonredundant protein and nucleotide data, and by computationally derived assertions including lipoprotein and transmembrane helix signatures. Assembled contigs greater than 5 kbp in length were assigned to chromosome or plasmid elements by a nucler alignment against the Acaryochloris marina strain MBIC11017 reference genome in the MUMmer package (Kurtz et al. 2004).

This whole-genome shotgun project has been deposited at DNA Data Bank of Japan/EMBL/GenBank under the accession AFEJ0000000. The version described in this paper is the first version, AFEJ01000000.

Identification and Analysis of Recent Duplicates

Paralogs within the genomes of Acaryochloris strains CCMEE 5410 and MBIC11017 were identified by local BlastP searches (Altschul et al. 1990) of each inferred protein sequence against its genome. Because the study was focused on the pools of recent duplicates, putative paralogs sharing less than 50% amino acid identity were removed from the data set. A similar search strategy was used to identify shared duplicates via reciprocal local BlastP searches. ORFs annotated as transposases, integrases, or identified as having significant homology (E < 0.05) to insertion sequence (IS) elements by BlastP against the IS Finder database (www-is.biotoul.fr/is.html) were also removed, as were gene families with more than ten paralogs (typically transposases).

Nucleotide alignments of duplicates were obtained by the manual adjustment of ClustalW automated alignments (Thompson et al. 1994) using the amino acid alignments as a guide. Silent site divergence (\(d_s\)) and replacement site divergence (\(d_{rs}\)) between aligned nucleotide sequences of duplicate pairs were estimated by the maximum likelihood (ML) procedure implemented in the codeml program of the PAML software package (version 3.14; Yang 1997). For all models, codon usage (the average nucleotide frequencies at the three codon positions) and transition/transversion bias were estimated from the data. Only duplicate pairs with \(d_s < 5\) were considered for further analysis.

Most cases involved a duplicate pair resulting from a single duplication event. For cases involving more than two paralogs, we used phylogenetics to distinguish the duplication events (e.g., resolution of three duplicates by reconstruction of the two duplication events). Phylogenies of aligned
nucleotide sequences were inferred by ML with PAUP* (Swofford 1996) according to a model of DNA sequence evolution selected by hierarchical likelihood ratio tests implemented by Modeltest (Posada and Crandall 1998). For the ML heuristic search, a starting tree was obtained by random sequence addition, and branch swapping was performed by tree bisection and reconnection. The resulting topology was used to specify the tree for the PAML model as described above.

**recA Phylogeny Reconstruction and Tests of Protein Adaptation**

Nucleotides (1,023) of the recA genes of *Acaryochloris* and other representative cyanobacteria were aligned by ClustalW (Thompson et al. 1994). A ML tree was reconstructed with PAUP* as described above according to the general time reversible (GTR) + I + G model of sequence evolution selected by Modeltest (Posada and Crandall 1998) and bootstrapped 100 times. A Bayesian analysis was performed with MrBayes (Huelsenbeck and Ronquist 2001) using the GTR + I + G model. Two independent chains of 1,000,000 generations of Markov chain Monte Carlo were analyzed, with trees sampled every 1,000 generations. Chain convergence was evaluated by the average standard deviation of split frequencies, and the first 20% of trees were discarded as burn-in. To test for the signature of positive selection during *Acaryochloris recA* diversification, branch-site models of codon evolution (Yang and Nielsen 2002) were implemented with PAML. Likelihood scores of nested models which either allow for a class of positively selected codon sites (i.e., $d_0/d_1 > 1$) or constrain $d_0/d_1$ to be less than or equal to 1 (the nearly-neutral model) were compared with a $\chi^2$ test. For branches of the recA tree for which the nearly-neutral model was rejected, a Bayes empirical Bayesian analysis was used to infer which codon sites belonged to the positively selected class with high (>95%) posterior probability.

**Results and Discussion**

**Acaryochloris Strain CCMEE 5410 Genome**

The *Acaryochloris* strain CCMEE 5410 genome was pyrosequenced to approximately 24× coverage depth, and the resulting genome data assembled into 511 contigs greater than 500 bp, with an N50 of 37,625 bp. The estimated genome size of 7.88 Mbp is somewhat smaller than that of the previously finished genome of *Acaryochloris* strain MBIC11017 (table 1; Swingley et al. 2008) as well as of a recently described strain isolated from the Great Barrier Reef for which an unpublished draft genome sequence has been obtained (~8.37 Mbp; Mohr et al. 2010) but is still considerably larger than those of other unicellular cyanobacteria. The strain CCMEE 5410 genome likewise contains fewer predicted ORFs than that of strain MBIC11017. The two genomes share similar base composition and a high number of ORFs with significant homology to IS elements (table 1).

The CCMEE 5410 and MBIC11017 genomes share 6,122 putative orthologs, with greater than 25% of predicted ORFs in each genome absent from the other (table 1). For the closed MBIC11017 genome, we can identify with certainty the genetic element on which each of these idiosyncratic ORFs resides. In addition to a circular chromosome, it contains nine apparently single-copy plasmids, varying in size from approximately 2.1 to 374 kbp, which together comprise roughly 22% of the genome (Swingley et al. 2008). For the CCMEE 5410 assembly, we provisionally assigned contigs greater than 5 kbp in length to either the chromosome or a plasmid element using a nucmer alignment against the MBIC 11017 genome (supplementary table S1, Supplementary Material online). This length cutoff was chosen because most short contigs either exhibited no homology to the MBIC11017 genome and/or encoded an IS element(s). One hundred and eighty-eight contigs totaling 5.81 Mbp were assigned to the chromosome, and 61 contigs with a cumulative size of 1.52 Mbp were assigned to plasmids (supplementary table S1, Supplementary Material online).

Gene content is generally conserved on the two *Acaryochloris* chromosomes. Approximately, 89% of ORFs on the MBIC11017 chromosome (5,621/6,342) have homologs in the CCMEE 5410 genome, whereas 83.5% of ORFs (4,951/5,932) on contigs assigned to the CCMEE 5410 chromosome have homologs in the MBIC11017 genome. Mapping of these chromosome contigs to the MBIC11017 reference indicated a high degree of sequence conservation and local synteny between chromosomes (fig. 1A; reference range data in supplementary table S1, Supplementary Material online).

By contrast, differences in gene content between the genomes are concentrated on plasmids. Seventy-seven percent (1,685/2,186) of MBIC11017 plasmid ORFs have no homolog in the CCMEE 5410 genome, accounting for 70% of the ORFs absent from the latter. The individual plasmids vary in the fraction of ORFs with homologs in the CCMEE 5410 genome from 0% (pREB9) to ~48% (pREB4). Similarly, for CCMEE 5410 contigs assigned to a plasmid, 55% of the 1,649 ORFs lacked a homolog in the MBIC11017 genome. In addition, few large blocks of synteny were observed among the MBIC11017 plasmid ORFs.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>CCMEE 5410</th>
<th>MBIC11017*</th>
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<tr>
<td>Genome size (Mbp)</td>
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<td>8.36</td>
</tr>
<tr>
<td>GC content (%)</td>
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<td>47</td>
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<tr>
<td>ORFs</td>
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<td>8,528</td>
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<tr>
<td>Strain-specific ORFs</td>
<td>2,261</td>
<td>2,406</td>
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<tr>
<td>IS elements</td>
<td>552</td>
<td>487</td>
</tr>
</tbody>
</table>

* Data from Swingley et al. (2008).
that were shared between the genomes (supplementary table S1, Supplementary Material online). The most extensive syntenic regions were clustered on plasmid pREB4 in a region spanning MBIC 11017 ORFs D0134–D0214 (fig. 1B). Blocks of synteny from this plasmid include genes responsible for the biosynthesis and maturation of a bidirectional hydrogenase (ORFs D0176–D0197; nucleotides 140334–159433) and a complete set of loci encoding an alternative ATP synthase (ORFs D0157–D0167; nucleotides 123957–132033). These results suggest a greater instability of the Acaryochloris plasmids compared with the chromosome.

Age Distribution of Duplicated Genes in Acaryochloris Genomes

Both genomes are notable for their large number of recent paralogs. We identified 393 and 597 duplicate pairs with synonymous-site divergence ($d_S$) less than $d_S = 5$ in the genomes of Acaryochloris strains CCMEE 5410 and MBIC11017, respectively. A majority of duplicated regions involve only a single protein-coding ORF; only ~29% of pairs ($N = 174$) in the strain MBIC11017 genome and ~35% of pairs ($N = 136$) in the strain CCMEE 5410 genome were a part of duplicated blocks of greater than one ORF. Most duplicates belong to the least divergent classes ($d_S < 1$; fig. 2A). The difference between strains in the observed number of duplicate pairs is primarily due to a greater number of duplicates in these classes in the genome of strain MBIC11017, which contains approximately double the number of duplicate pairs with $d_S < 0.5$ (278 vs. 143). By contrast, the number of duplicate pairs with $d_S > 2$ is similar between the genomes (121 vs. 102). For both Acaryochloris genomes, the number of duplicate pairs with $d_S < ~1.5$ is very large compared with other representative bacterial genomes (fig. 2B; Hooper and Berg 2003b). For greater levels of $d_S$, duplicate numbers are comparable, with the exception of an apparent enhanced density of duplicates in Acaryochloris genomes centered on $d_S$ values of ~2–2.4 (fig. 2).

Most duplicate pairs from the least divergent classes are strain specific, whereas more divergent duplicates are
generally more likely to be present in both genomes (fig. 3). This pattern is in accord with the expectation that silent site divergence is generally a reasonable proxy for the age of a duplication event and that less divergent duplicate pairs have therefore largely originated following the divergence of these strain lineages from their common ancestor. However, there are a number (N = 60) of low divergence (\(d_S < 1\)) duplicate pairs in the strain CCME 5410 genome that also are found in the strain MBIC11017 genome. Such pairs may be the result of convergent duplication events following strain divergence or, alternatively, may appear “younger” than they are due to either gene conversion or extreme sequence conservation at synonymous sites. We believe that slower than average evolutionary rates is of primary importance for these loci because clear evidence from phylogenetic analyses for either convergent evolution or gene conversion (i.e., paralogs clustering by strain) was observed for only a minority (N = 14) of duplicate pairs (data not shown). Among more divergent duplicates, approximately 50% (77/166) of duplicate pairs of divergence level \(d_S > 1\) in the strain CCME 5410 genome are present in the MBIC11017 genome (fig. 2). The unique duplicates among the more divergent age classes suggest that there has been the differential retention of older duplicates between genomes following strain divergence.

**Estimation of Duplicate Birth and Death Rates**

Following the approach of Lynch and Conery (2000, 2003), we modeled each age distribution as a steady-state birth–death process in order to estimate the rates at which duplicates arise and disappear from the respective Acaryochloris genomes. Because the assumption of constant birth and death rates is more likely to be valid over a short time scale, we limited the analyses to duplicate pairs with silent site divergence less than \(d_S = 0.1\). For both data sets, we also excluded duplicate pairs in these age classes (N = 7 pairs) found in both genomes (see above) to remove the potential impacts on the analysis of either gene conversion events or slowly evolving duplicates. We note that similar results were obtained for the full data set (not shown).

Under a steady-state birth–death process, the instantaneous rate of removal of duplicates from the genome (\(d\)) can be estimated by the slope of the linear regression of ln \(n_i\) on \(d_S\), where \(n_i\) is the number of duplicate pairs in age class \(i\). The regression models explained most of the variation in both data sets (\(R^2 = 0.82\), \(P < 0.0001\) for Acaryochloris strain CCME 5410; \(R^2 = 0.76\), \(P < 0.0001\) for Acaryochloris strain MBIC11017), suggesting that the assumption of constant birth and death rates over this time interval is reasonable. Estimates of \(d\) (standard error [SE]) were not significantly different for the two strains: 8.0 (2.14) for CCME 5410 and 7.8 (2.52) for MBIC11017. This corresponds to estimated half-lives (scaled to synonymous site divergence) of 0.087 and 0.089 for Acaryochloris strains CCME 5410 and MBIC11017, respectively. That is, most duplicates are expected to be lost rapidly from the genome. These values are within the range observed among eukaryotic genomes (Lynch and Conery 2003).

We estimated the duplicate birth rate \(B\) (the probability that a gene duplicates over the divergence period \(d_S = 0.1\)) for each genome by \(B = (n_Sd \times d_S)/N (1 - e^{-d} \times d_S)\), where

![Fig. 2](image-url) --- (A) Frequency distributions of duplicate pairs for Acaryochloris strains MBIC11017 (blue) and CCME 5410 (green). (B) Frequency distributions of Acaryochloris duplicate pairs compared with data for Escherichia coli K12, Bacillus subtilis 168, and Pseudomonas aeruginosa PA01 (from Hooper and Berg [2003b]).

**Fig. 3.** --- Fraction of duplicates of different divergence levels in the Acaryochloris CCME 5410 genome that are shared with the strain MBIC11017 genome.
ever, most young duplicates as well as those which have been retained over longer periods appear to be under strong purifying selection against protein change: the median of $d_N/d_S$ in the $d_S < 0.1$ divergence level classes is $\sim 0.2$ and $\sim 0.3$ for the strain MBIC11017 and strain CCME 5410 genomes, respectively. For duplicates of divergence level greater than $d_S = 1$, mean (SE) value of $d_N/d_S$ is 0.12 (0.004) for the strain MBIC11017 genome and 0.09 (0.004) for the strain CCME 5410 genome. Bearing in mind that the estimated strength of constraint represents the cumulative history of selection since duplication, this pattern indicates that, on average, the intensity of purifying selection on duplicates increases over time. We conclude that the period of near-neutral evolutionary dynamics is at most brief following gene duplication, applies to only a subset of duplicate pairs, and usually is followed by either purging from the genome or an increase in selection against protein change. These results are similar to those obtained for other bacteria (Hooper and Berg 2003b) as well as for eukaryotic genomes (Lynch and Conery 2000, 2003; Aury et al. 2006).

**Physical Location of Duplicated Genes**

The location of duplicates at (or near) the time of birth may provide clues regarding the substrates and prevailing mechanisms responsible for duplicate formation. Few duplicates ($\sim 3\%$ of duplicate pairs) are in tandem (operationally defined here as being within five ORFs of each other) at present in either *Acaryochloris* genome.

The closed genome of *Acaryochloris* strain MBIC 110107 enabled a comprehensive investigation of the distribution of duplicates on the chromosome and on extrachromosomal elements, respectively. For the least divergent classes, at least one gene copy resides on a plasmid for most duplicate pairs (fig. 5A and B), with both on plasmids for greater than $60\%$ of duplicates with a synonymous divergence level of $d_S < 0.5$. Because duplicates might move over time, the locations of the least divergent duplicates are expected to be most representative of where they originated. Of the 133 duplicate pairs of divergence level $d_S < 0.1$, both members are found on the same genetic element (chromosome or plasmid) only $\sim 16.5\%$ of the time. Similarly, 13 of 21 identical (i.e., $d_S = d_H = 0$) duplicate pairs are located on different elements, and of the eight which are on the same element, six likely originated as part of the same duplication event on plasmid pREB3. The origin of most duplicates therefore appears to involve recombination between different plasmids (67/133) or between a plasmid and the chromosome (44/133).

Chromosome–chromosome pairs make a substantial contribution to the pool of duplicates from more divergent classes, however, with $40\%$ of duplicates both residing on the chromosome at divergence levels greater than $d_S = 2$ (fig. 5C). Plasmid–plasmid duplicates are nearly absent in...
Although most gene duplication events involve interplasmid or plasmid–chromosome exchange, it therefore appears that the vast majority are destined for loss from the genome. Duplicates that are retained over the long term tend to either originate on the chromosome or end up there.

We reach a similar conclusion for the CCMEE 5410 genome (supplementary fig. S1, Supplementary Material online), although we could not assign one or more copies of a duplicate pair to a genetic element for 25% of duplicates. Most of these unassigned pairs belonged to low divergence classes (dS < 1.0), with one-third from a divergence class of dS < 0.1. Inability to resolve the locations of these duplicates was due to the presence of one or both copies on a short contig and is likely responsible for the observed lower than expected density of interplasmid and plasmid–chromosome pairs in these low divergence classes (supplementary fig. S1 A and B, Supplementary Material online; compare with fig. 2A). The placement on short contigs suggests that they are flanked by repetitive DNA (including IS elements) that may have served as substrates for recombination.

**Duplicate Retention**

Bacterial genomes may exhibit a biased retention of duplicates from different gene functional classes (Gevers et al. 2004). Analysis of the strain CCMEE 5410 genome indicated differences in the likelihood of retention among clusters of orthologous groups (COGs) functional classes (fig. 6). In particular, the pool of duplicated genes (dS < 5) is enriched in members from the transcription (K), carbohydrate transport and metabolism (G), ion transport and metabolism (P), signal transduction (T), and unknown (S) functional classes compared with their genome-wide frequencies. Conversely, there is a general paucity of duplicated genes involved in translation (J), replication, recombination and repair (L), cell wall/membrane/envelope biogenesis (M), amino acid transport and metabolism (E), and coenzyme transport and metabolism (H). This suggests that gene dosage balance may generally be more critical within these classes, with duplication of individual genes strongly selected against.

The observed biased retention of recent gene duplications in the G, K, and P classes, as well as a deficiency of H, J, and M classes, is in accord with general longer term evolutionary trends revealed for paralogous gene family expansion in a survey of 48 bacterial genomes (Gevers et al. 2004). The retention of signal transduction (T) and transcription factors (K) is also a feature of plant genomes following polyploidization (Blanc and Wolfe 2004; Maere et al. 2005; Chapman et al. 2006; Thomas et al. 2006).
Unique duplicates retained by the respective genomes may confer environment-specific fitness benefits through dosage effects, a phenomenon frequently observed in laboratory populations of bacteria (Roth et al. 1996; Romero and Palacios 1997; Reams and Neidle 2003). A correlation between duplicate content and environment has also been observed for yeast (Ames et al. 2010). The genome of strain MBIC11017 possesses a striking number of duplicated genes involved in nutrient acquisition (principally the binding, transport, and metabolism of iron) that exist as either single copies or are not found in the strain CCMEE 5410 genome (table 2). All but one of these include a plasmid-encoded duplicate copy. We note that the strain MBIC11017 genome also includes eight plasmid-encoded single-copy iron acquisition genes that are absent from the strain CCMEE 5410 genome (ORFs A0156, A0157, A0172, A0197, A0198, A0274, B0123, B0125).

That this strain’s genome may have been shaped by iron limitation is also suggested by the recent duplication of the light antenna protein $\text{pcbC}$ (table 2). This gene is upregulated by $\text{Acaryochloris}$ cells under conditions of iron deficiency (Chen et al. 2005), and PcbC protein subunits produce a light-harvesting antenna for photosystem I that may compensate for the reduction in the level of this photosystem relative to photosystem II that occurs during iron stress.

Tropical Pacific waters generally appear to be low in iron (e.g., Coale et al. 1996; Behrenfeld et al. 2006). Although we do not know the iron concentration of the local environment from which strain MBIC11017 was isolated, there are reasons to believe that $\text{Acaryochloris}$ cells may be iron limited in their natural habitat. This strain was isolated from underneath the ascidian, $\text{Lissoclinum patella}$ (Miyashita et al. 1996), which belongs to a suborder (Aplousobranchia), which includes members notable for the accumulation of high concentrations of iron from the environment in blood cells called ferrocytes (Endean 1955). In addition, the positive response of MBIC11017 laboratory cultures to heavy iron addition suggests an organism with high demand for this nutrient (Swingley et al. 2005).

Other recent duplicates in the MBIC11017 genome that are involved in light-harvesting encode pigment and scaffold components of phycobiliproteins (table 2), the major accessory pigments in photosynthesis for most cyanobacteria. Multiple duplicate copies of genes for the phycobiliprotein phycoerythrin, which specifically harvests yellow–orange light for photosynthesis, as well as linker proteins essential for the assembly of phycobiliprotein rods, are located on plasmid pREB3 (Swingley et al. 2008). Strain MBIC11017 produces phycobiliproteins under low light conditions in the laboratory (Chan et al. 2007). By contrast, strain CCMEE 5410 does not produce phycobiliproteins (Chan et al. 2007), and these genes are missing entirely from its genome (table 2). This pattern suggests differences in the availability of yellow–orange light in the two environments. These wavelengths appear to be available at low levels in the natural environment of strain MBIC11017 (Kühl et al. 2005), whereas they may be more rapidly attenuated in the turbid Salton Sea environment from which strain CCMEE 5410 was

**Table 2**

<table>
<thead>
<tr>
<th>ORFs*</th>
<th>Annotation</th>
<th>$d_s$</th>
<th>CCME 5410</th>
</tr>
</thead>
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<tr>
<td>0473/A0147</td>
<td>Fe$^{2+}$-transporter $\text{feoB}$</td>
<td>0.26</td>
<td>7582</td>
</tr>
<tr>
<td>0474/A0146</td>
<td>Fe$^{2+}$-transporter $\text{feoA}$</td>
<td>0.11</td>
<td>7581</td>
</tr>
<tr>
<td>3038/(B0139/F0079)</td>
<td>Fur transcriptional regulator</td>
<td>0.55/0.30</td>
<td>2939</td>
</tr>
<tr>
<td>3040/F0079</td>
<td>Putative Fe$^{3+}$-transporter</td>
<td>0.21</td>
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</tr>
<tr>
<td>3348/A0161</td>
<td>Fe$^{3+}$-dicitrate ABC transporter</td>
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<tr>
<td>3403/A0184</td>
<td>TonB-dependent siderophore transporter</td>
<td>0.15</td>
<td>0729</td>
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<tr>
<td>3416/A0185</td>
<td>Ferrichrome ABC transporter</td>
<td>0.31</td>
<td>0738</td>
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<tr>
<td>C0108/C0205</td>
<td>Heme oxygenase (Fe-recycling)</td>
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<td>1368/3655</td>
<td>Iron deficiency light antenna $\text{pcbC}$</td>
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<td>—</td>
</tr>
</tbody>
</table>

* ORFs on plasmids are preceded by a letter indicating plasmid identity.
isolated (Miller et al. 2005) by phycobiliprotein-producing plankton (Wood et al. 2002) and inorganic particulate matter (Swan et al. 2007) in the overlaying water column.

The Salton Sea is a phosphorus-limited system characterized by high concentrations of dissolved organic carbon and nitrogen (Schroeder et al. 2002), as well as of iron, primarily as reduced particulates (Holden and Montañé 2002; de LeBlanc and Schroeder 2008). The unique du-
selenium, and zinc) concentrations are also high (Vogl and Koff et al. 2008). Heavy metal (including cadmium, copper, and Roth 1979), particularly for long recombining sequen-
cases such as IS elements (but see Reams et al. [2010], e.g., in which duplication depends only weakly on homologous recombination). The large number of IS elements in Acaryochloris genomes (table 1) provide potential substrates for recombination. Although there appears to be a general trend against the retention of duplicates involved in DNA replication, recombination, and repair (fig. 6), both genomes contain a number of duplicated genes from this functional class, and we briefly consider here whether these duplicates may play a role in the enhanced duplication dynamics of these genomes.

Most notably, there are an unusually large number of recA copies in both Acaryochloris genomes. RecA is a multi-
functional protein that is central to homologous recombination, is involved in recombination-mediated DNA damage repair and rescue of stalled replication forks, is required for mutagenesis mediated by translesion synthesis, and regulates gene expression through its coprotease activity (reviewed by Miller and Kokjohn 1990). The strain MBIC11017 genome contains seven recA copies (Swingley et al. 2008), whereas there are four complete copies in the genome of strain CCME 5410. The CCME 5410 genome also includes a truncated copy (ORF 6290) with a nonsense mutation at codon 241 produced by an apparent transposition event that results in the loss of part of the ATPase core and the C-terminal domain; the putative 3’ end of the gene copy is found on a different contig (ORF 8203) and is also adjacent to a transposase. In contrast, recA exists as a single copy in the vast majority of bacterial genomes; the only known exceptions are the Acaryochloris genomes and those of Myxococcus xanthus (two copies; Norioka et al. 1995), Bacillus megaterium (two copies; Nahrstedt et al. 2005), and Deinococcus deserti (three copies; de Groot et al. 2009).

<table>
<thead>
<tr>
<th>ORFs*</th>
<th>Annotation</th>
<th>ds</th>
<th>MBIC11017</th>
</tr>
</thead>
<tbody>
<tr>
<td>0720/1772(2355/2491)</td>
<td>Fructose-bisphosphate aldolase</td>
<td>2.591.48</td>
<td>3372</td>
</tr>
<tr>
<td>2488/2356</td>
<td>XuF5/ Fru6P phosphoketolase</td>
<td>2.692.27</td>
<td>0443</td>
</tr>
<tr>
<td>1774/2356</td>
<td>Acetate kinase</td>
<td>2.57</td>
<td>0445</td>
</tr>
<tr>
<td>2357/2490</td>
<td>Phosphoglycerate mutase family</td>
<td>1.58</td>
<td>—</td>
</tr>
<tr>
<td>2374/2465</td>
<td>Phosphoglycerate mutase family</td>
<td>1.03</td>
<td>—</td>
</tr>
<tr>
<td>2365/2496</td>
<td>Putative glycogen phosphorylase</td>
<td>0.84</td>
<td>—</td>
</tr>
<tr>
<td>2349/2468</td>
<td>Cu resistance protein CopA</td>
<td>0.31</td>
<td>—</td>
</tr>
<tr>
<td>2354/2487</td>
<td>Copper-translocating ATPase</td>
<td>1.10</td>
<td>—</td>
</tr>
<tr>
<td>2372/2481</td>
<td>Copper-translocating ATPase</td>
<td>1.21</td>
<td>—</td>
</tr>
<tr>
<td>3189/7004</td>
<td>RND family multidrug efflux</td>
<td>2.22</td>
<td>2480</td>
</tr>
<tr>
<td>1784/6258</td>
<td>RND family multidrug efflux</td>
<td>0.48</td>
<td>0454</td>
</tr>
<tr>
<td>2586/2489</td>
<td>Glutaredoxin</td>
<td>1.690.84</td>
<td>3463</td>
</tr>
</tbody>
</table>

* ORFs assigned to plasmids are italicized.
**Escherichia coli** exhibits a 10-fold or greater tandem duplication rate if RecA is constitutively activated (Dimpfl and Echols 1989), and overexpression of its eukaryotic homolog RAD51 may also enhance duplication rate as well as generally increase genome instability (reviewed by Klein 2008). Whether the greater recA copy number in *Acaryochloris* genomes results in enhanced expression remains to be determined, but the association between copy number and strain duplication rate is consistent with a dosage effect. Also consistent with this possibility, the *D. deserti* genome likewise appears to contain a greater number of paralogs (100–200) than those of its single-copy congeners, *D. radiodurans* and *D. geothermalis* (de Groot et al. 2009).

*Acaryochloris* recAs are both extremely diverse and monophyletic, indicating that this diversity likely originated solely during *Acaryochloris* diversification rather than by horizontal gene transfer (HGT) (fig. 7). Three chromosomal copies are shared by the strains and appear to predate divergence from their common ancestor, whereas the strains vary in the number of plasmid-borne copies. Although on average all copies have experienced strong purifying selection ($d_{s}/d_{a} = 0.05$), there is some evidence that certain amino acid substitutions have been selectively favored during recA diversification. Along two branches (labeled A and B in fig. 7), branch-site models of codon evolution (Yang and Nielsen 2002) which allow for positive selection on one or a few codon sites on specific branches of a phylogeny had significantly greater likelihood values than nearly-neutral models constrained to $d_{s}/d_{a}$ ≤ 1 for all codons ($2ΔL = 70.42$, $P = 0$ for the Branch A model; $2ΔL = 9.16$, $P = 0.01$ for the Branch B model). The codons estimated to have experienced positive selection (i.e., $d_{s}/d_{a} > 1$ with a posterior probability $P > 0.95$ by Bayesian analysis) at some point during recA diversification (supplementary fig. S2; Supplementary Material online) include sites that participate in monomer–monomer interactions in the RecA filament (codons 105, 114, 115, 127, 153, and 240), make contact with ssDNA-binding sites (codon 153), or change the properties (e.g., charge) of the C-terminal domain of the protein (codons 312, 323, and 328), which is known to autoregulate RecA activity and to bind dsDNA during homologous recombination (Cox 2007). Whether these changes have consequences for RecA structure and function remains to be investigated, as does the possibility that diversification has yielded paralogous RecAs with nonredundant functions (i.e., subfunctionalization) in *Acaryochloris* cells.

**Concluding Remarks**

Strain-specific duplicates concentrated on plasmids make a substantial contribution to gene content differences
between Acaryochloris genomes and appear to be selectively retained in their respective contemporary environments by favorable dosage effects. These differences are in part the product of the differential retention of duplicates of chromosomal origin (fig. 4B; see below). The lower degree of conservation of gene content on plasmids compared with the chromosome also suggests an important role for HGT in Acaryochloris evolution. If this is the case, the implication is that the ultimate source of many duplicate pairs is a single-copy gene of foreign origin. In Proteobacteria and Firmicutes, horizontally transferred genes do appear to be more likely to be duplicated (Hooper and Berg 2003a).

To obtain a conservative estimate of the contribution of HGT to the pool of strain-specific duplicates with at least one copy on a plasmid, we performed BlastP analyses against the NCBI Blast nonredundant protein database for each genome. For a given strain-specific duplicate family without an ortholog in the other strain, it can be difficult to unequivocally determine whether it is the product of the differential retention of an ancestral gene or of HGT. This is because many of these loci either exhibit greatest sequence similarity to a different cyanobacterium or have no similarity to another sequence in the database (i.e., are orphan genes). Therefore, taking a conservative approach and using an E cutoff value of $10^{-20}$, we considered a duplicate family to be of vertical origin if the top non-Acaryochloris hit for a duplicate family was a cyanobacterium, to have originated by HGT if the top hit was another taxon and to be of unknown origin if it was an orphan.

For duplicate pairs for which one copy is on the chromosome (fig. 4B), most are inferred to be of cyanobacterial origin in both Acaryochloris genomes by the above criteria (62% for strain MBIC11017 and 77% for the subset of duplicates in strain CCME 5410 which could be fully assigned to genetic elements). This is the expectation if the plasmid copy was derived by duplication of a chromosomal template. Fewer duplicates in this category appear to involve horizontally transferred loci (4% and 3%, respectively). For interplasmid duplicates, however, a larger fraction contains duplicates with homology to a family of Rieske-FeS motif-containing oxygenases involved in chlorophyll synthesis and degradation (ORFs 0307/5640 in CCME 5410 and 0159/A0067 in MBIC11017). It is notable that A0067 is found within one of the few regions of extensive synteny between a MBIC11017 plasmid and the CCME 5410 genome (including A0036–A0053 and A0066–A0075). Whether one of these paralogs has diverged to specifically degrade Chl d awaits further investigation.

Supplementary Material

Supplementary table S1 is available at Genome Biology and Evolution online (http://www.gbe.oxfordjournals.org/).

Acknowledgments

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