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The Historical Biogeography of Phototrophic Consortium

Emma Ciechanowski

Western Washington University, emma.ciechanowski@wwu.edu

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the
HISTORICAL
BIOGEOGRAPHY
of PHOTOTROPHIC
CONSORTIUM

BY EMMA CIECHANOWSKI

INTRODUCTION

A consortium is a close physical association between microbial cells of different lineage (Liu et al. 2013). The phototrophic consortium is an aggregation of two different lineages of bacteria: one large motile bacterium surrounded by many smaller photosynthetic bacteria. They live in freshwater lakes around the world with varying degrees of morphological differences. Bacteria are historically accepted as a ubiquitous species, meaning that if the environment permits them to live there, they will. This hypothesis was first introduced by Beijerinck and Becking in the early 1900s to describe the distribution of microorganisms, with the official mantra being, "everything is everywhere, but the environment selects" (Becking 1934, 15). Although this has proven true with common pathogenic bacteria such as *E. coli* and *Salmonella*, studies suggest that conditions do not always permit this hypothesis to hold true.

With the similarities in environment that the phototrophic consortia inhabit, and the striking parallels between the evolved interspecific interactions, it would make sense that they are all descendants of one symbiotic partnership, and have diverged morphologically over time within a region. However, the morphological differences that have been found to exist between the geographic distributions of bacteria may indicate separately evolved symbiotic relationships. I believe that the increased fitness—greater ability to survive and divide—provided by the consortium of the two bacterial genera was enough to drive the same symbiotic evolution across separate geographic locations.

SYMBIOTIC INTERACTION

Interspecific interactions have the ability to increase the fitness of one or both organisms. These interactions come in a variety of flavors, with the differences being whether one or both of the organisms are benefiting from the interaction. Interspecific interactions also have varying degrees of proximity, from living completely separate from each other and interacting sparsely, to living inside of one another. When one organism lives in close contact with another, it is called a symbiosis. This close association between organisms allows for a powerful reciprocal selection to occur, resulting in coevolution. This is one of the more beautiful artifacts of evolution because through cooperative symbiosis, the throes of time, and the environment, the resulting organisms—by varying degrees—need each other. For bacteria, this is not an uncommon trait. One example is the rumen bacteria possessed by cows, which live in the digestive tract and help digest the consumed plant matter in exchange for nutrients. Interbacterial mutualisms are common with varying degrees of codependence. Many bacteria are able to thrive in wildly diverse communities, utilizing important metabolites from the metabolic waste of others. In layman's terms, one man's trash is another man's treasure. The phototrophic consortium that I will address here is exemplary of a mutually beneficial symbiotic relationship.

ECOLOGY, MORPHOLOGY AND PHYSIOLOGY

Since their discovery over one hundred years ago, there have been seven described morphologies of the phototrophic consortium; all are made up of a single, large, heterotrophic motile bacterium, covered by 13–69 non-motile, phototrophic sulfur bacteria, also called epibionts (Madigan 2015). The central bacterium is motile due to the presence of a monotricious flagellum, though some also have gas vesicles, which allow it to move vertically in the watercolumn (Table 1). The epibionts are either phototrophic green or brown sulfur bacteria, or in one reported case, a combination of both (Table 1).

PHOTOTROPHIC CONSORTIUM

Phototrophic consortia are found worldwide in freshwater lakes and ponds where light reaches areas of water containing hydrogen sulfide (Madigan 2015). In these lakes, the consortia are constantly repositioning in order to maintain adequate light levels for photosynthesis and sulfur metabolism.

Movement of the motile central bacterium directly benefits the non-motile epibionts. The phototrophic consortium differs from motile phototrophic bacteria in that they move away from the light as opposed to towards it, in an exhibition of *scotophobotaxis* (Hays et al. 2015). The phototropic consortium also exhibits chemotaxis towards sulfide and sulfide-containing compounds (Liu et al. 2013). This movement is significant in understanding the symbiosis because the bacteria doing the movement is neither phototrophic, nor is it a sulfur bacteria. Movement done by the central bacteria is to serve the needs of its phototrophic sulfur-loving passengers. The mutual benefit of the symbiosis between the two is a ride in exchange for some food.
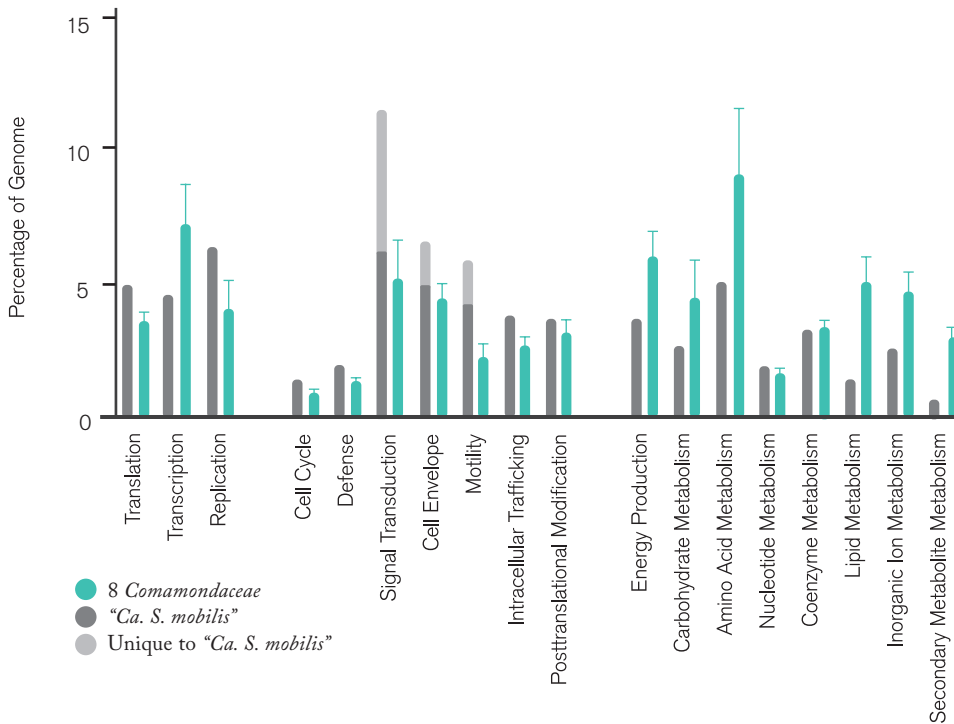
FIGURE 1.

A comparison of the gene contents of the central bacterium of the phototrophic consortium *"Ca. S. mobilis"* and eight of its non symbiotic relatives. Green bars indicate the average percentage of the genome belonging to the eight relatives, gray bars indicate the percentage of the genome belonging to *"Ca.S. mobilis"*, and light gray bars indicate the percentage of the genome that is unique to the *"Ca.S. mobilis"* (Liu et al, 2013).

In order to understand the biogeographic relationship between the different populations of the phototrophic consortium and their evolution, it is important to understand the depth of their symbiosis; the phototrophic consortium represents one of most interdependent relationships between two unrelated bacteria. They have melded many of their physiological processes, are metabolically coupled, and have coordinated their cell division. Even the names of the organisms, which are purely taxonomically based, are put in quotation marks due to the fact that they are a mixed culture and not a true species (Hays et al. 2015). In fact, the central bacterium is likely no longer capable of independent growth; so far, it has been impossible to independently culture without the help and essential metabolites from its phototrophic partner. This is due to a massive gene loss during its symbiotic evolution, especially for genes involved in metabolism (Liu et al. 2013). An example of this gene loss is in the *"Candidatus Symbiobacter mobilis"* (*"Ca. S. mobilis"*), the central bacterium from the most studied consortium, *"Chlorochromatium aggregatum."* The lost genes were identified by comparing eight different non-symbiotic genomes from the *Comamonadaceae* family with that of the *"Ca. S. mobilis."*

In addition to losing genes, the *"Ca. S. mobilis"* also gained some that were not found in the other eight *Commanonadaceae* genomes (Liu et al. 2013). These genes were mainly involved in signal transduction, cell envelope biogenesis, and cell motility (Figure 1). This is likely due to the increased need for intercellular communication and motility, and is indicative of a long-term coevolutionary relationship. Due to the high specificity between the bacteria, it would seem more probable that the symbiosis evolved once, rather than multiple times, supporting the hypothesis of a symbiotic ancestor followed by distribution. However, short generation times increases the speed of evolution in bacteria. If the symbiosis is highly beneficial and dramatically increases fitness, it is possible that the phototrophic consortium evolved separately, multiple times.

SLOW CLOCK ANALYSIS OF rRNA

Depending on the protein or molecular marker being examined, phylogenies can give differing relative estimates of time. Based on the protein being examined, one can look at interrelatedness based on "fast clock" or "slow clock" analysis. The less essential a gene is, the more likely it is to be a fast clock gene. Its ability to accumulate mutations and evolve is increased because it is less constrained by purifying selection. Slow clock genes are more ancient and essential; they withstand mutations due to higher levels of purifying selection and thus have higher levels of conservation.

Ribosomal RNA (rRNA) is often used to analyze interspecific relatedness for many reasons. First, it exists in all living organisms, which indicates that it is highly conserved; this makes the variable regions much more significant. Second, it is not involved in horizontal gene transfer, which is important because we can definitively say that the phylogenetic relationships are due to ancestry— or lack thereof—and not part of a randomly acquired gene. Third, there is already a rather large database, so the comparison and sequence alignment of rRNA with other species and organisms is easy to perform, and can give a wider picture of relatedness without having to experimentally examine an entire organism's family.

ANALYSIS OF EPIBIONT rRNA

The phylogenetic relationships between the different "species" in different lakes of epibionts were investigated by analyzing the 16S rRNA. This was completed in a 2000 study by Fröstl and Overmann. The study investigated the relationship between the epibionts of *"C. aggregatum"* from Lake Dagow in Germany and the epibionts of *"C. aggregatum,"* *"C. glebulum,"* and *"C. magnum"* from Lake Echo in Washington, U.S.A. So far only the epibionts have been able to be cultured, due to the high sensitivity and dependence of the central bacterium (Stewart 2012). Comparative analysis of the 16S rRNA was done through PCR amplification and sequencing, followed by sequence alignment. The 16S rRNA gene fragments of the different sulfur bacteria

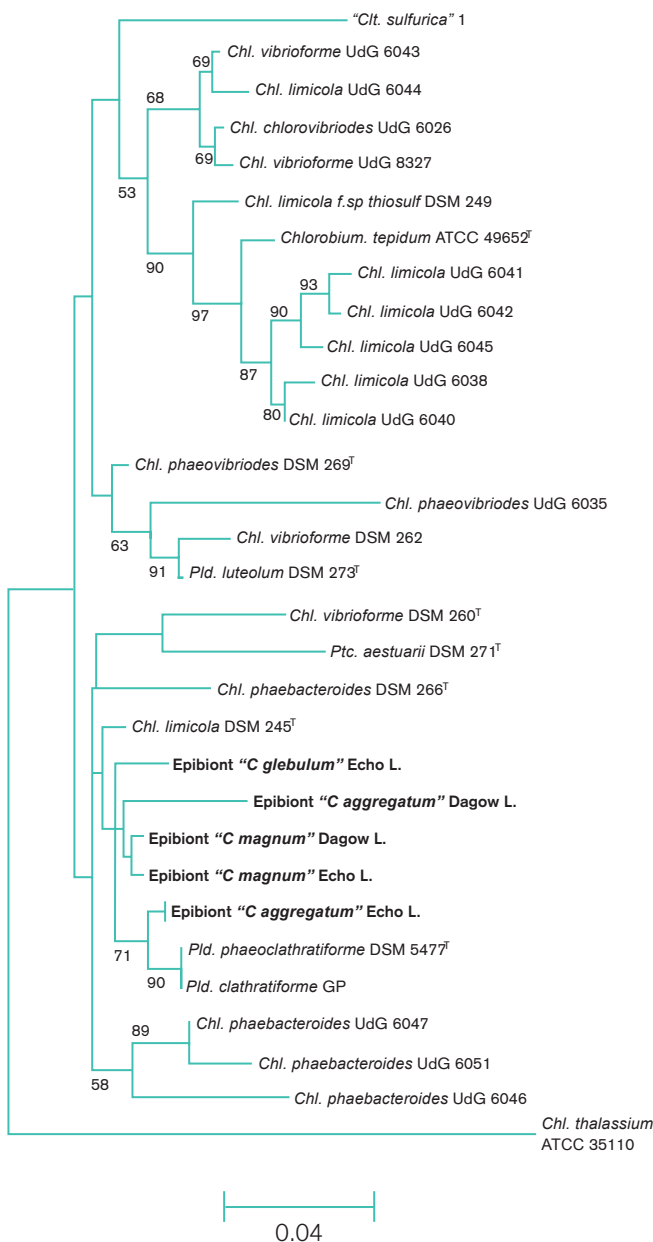SHORT GENERATION TIMES INCREASES THE SPEED OF EVOLUTION IN BACTERIA

FIGURE 2.

A phylogenetic tree of the five different epibionts from Lake Dagow and Lake Echo, along with known radiations of non-symbiotic green sulfur bacteria. The bar at the bottom indicates 0.04 fixed point mutations per base (Fröstl and Overmann, 2000).

were analyzed by denaturing gradient gel electrophoresis, which yielded single, unambiguous bands (Fröstl and Overmann 2000).

Fröstl and Overmann's study found that the banding analysis of the denaturing gradient gel in the 16S rRNA sequence found that the epibionts from *"C. aggregatum," "C. glebulum,"* and *"C. magnum"* from Echo Lake had high similarity compared to the epibionts *"C. aggregatum"* and *"C. magnum"* from Dagow Lake (Fröstl and Overmann 2000). This showed that cells of a specific morphotype of consortia living in a single lake belong to the same phylotype.

Phylogenetic analysis of the 16S rRNA found that the epibionts form a cluster in the sulfur bacterial radiation with six other free-living strains (Fröstl and Overmann 2000). This analysis supports the idea that although they are closely related, there is no singular ancestral appearance of the symbiotic trait (Figure 2). Additionally, the phylogenetic analysis of the epibionts from the consortia of Lake Dagow and Lake Echo showed that similarity in location was not tied to higher levels in relatedness (Figure 2). This supports the hypothesis that the symbiosis of the phototrophic consortium evolved multiple times in multiple locations.
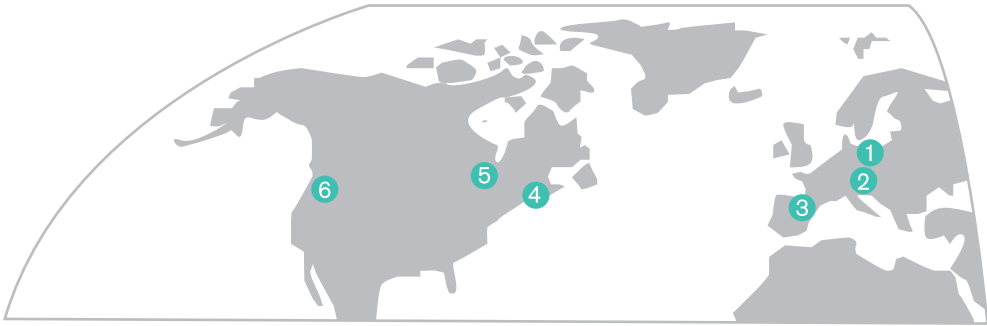
FIGURE 3.
This is a map of the geographic regions where samples of phototrophic consortia were taken. Region 1 and 2 sampled 3 different lakes in Germany. Region 3 sampled two different lakes in Spain. Region 4 sampled one pond in Massachusetts. Region 5 sampled seven lakes in Michigan. Region 6 sampled one lake in Washington (Glaeser and Overmann, 2004).

Another study by Glaeser and Overmann in 2004 examined the phylogenetic relatedness between epibionts of phototrophic consortia from 14 lakes with similar environmental parameters in six different geographical regions: Germany, Spain, Massachusetts, Michigan, and Washington (Figure 3). Like Fröstl and Overmann's study in 2000, this study also used the 16S rRNA as a genetic marker in order to determine the phylogenetic relatedness between the different morphotypes and strains of the epibionts. Out of the seven morphological forms of phototrophic consortia found in the 14 lakes, 15 distinct 16S rRNA sequence types were detected (Glaeser and Overmann 2004). To complicate matters, these distinct 16S rRNA sequence did not always match with specific morphological forms (Table 2). This resulted in a total of 19 different "phylomorphotypes," a term coined to describe the different combinations of morphology and phenotypes (Table 2).

It was found that in a single lake, the matches between phylotype and morphotype would be consistent, whereas on a global scale they were not. The distinct phylogenetic clusters of the phototrophic consortium suggest that the symbiosis either arose independently, or that a common ancestor of the sulfur bacteria was symbiotic. If the latter were the case, then the free-living trait would be the one to have evolved independently, which is unlikely due to the widespread nature of the green sulfur bacteria radiation. The phylogenetic analysis of the 16S rRNA over the six geographic regions showed that there is a nonrandom pattern of

THEREFORE, THE PHOTOTROPHIC CONSORTIUM HAS NOT DESCENDED FROM A SINGLE ANCESTOR.

distribution, with increased differences across continents (Glaeser and Overmann 2004). Therefore, the phototrophic consortium has not descended from a single ancestor.

DISCUSSION AND FINAL THOUGHTS

It has been assumed that microorganisms do not have geographical barriers for distributions, and that given high levels of ubiquity in microorganisms, and a suitable environment, a microorganism will live there. The study of the biogeographic distribution of phototrophic consortia shows us the flaws in these assumptions. There is a nonrandom distribution and little similarity in the epibiont of phototrophic consortia across continents, and higher similarity in populations in neighboring lakes. This provides evidence for a slow dispersal of the phototrophic consortia over large distances. However, due to the nature of microorganisms, the speed and ingenuity of their evolutions make it possible for complicated traits to arise independently in populations. The close association of consortia expands the

functional and metabolic abilities of the organisms and allows for a greater ability to survive perturbation, thus increasing the fitness of both organisms (Hays et al. 2015). This increased fitness means that there is a greater likelihood of symbiotic traits becoming fixed in a population. Considering both of these factors and the phylogenetic data from recent studies, I believe that the phototrophic consortium evolved separately across large spatial distances, but experienced dispersal across short spatial distances.

Research on the origins and basis of symbiotic interactions between bacteria is important for an understanding of the wide range of mutualism that exists in microbial communities. The consequence of mutualisms between bacteria is not only beneficial for the bacteria themselves, but also for the stability of ecosystem function. Nestled at the bottom of the food chain, microorganisms are the backbone to any ecosystem. Just as genetic diversity within a population improves that species' adaptability and chance of survival, diversity within the microbial community of an ecosystem helps serve as a buffer for environmental perturbations. Phototrophic consortia are not only of ecological significance in lake ecosystems, but are also excellent model systems for the evolution of the bacterial interactions. Studying their intimate and complex symbiotic relationship helps give us a better understanding of the molecular mechanisms and evolutionary factors that may have led to multicellularity, and hence the diversity of eukaryotic life we see on Earth today.

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