The Green Algal Underground: Evolutionary Secrets of Desert Cells

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Microscopic, unicellular, free-living green algae are found in desert microbiotic crusts worldwide. Although morphologically simple, green algae in desert crusts have recently been found to be extraordinarily diverse, with membership spanning five green algal classes and encompassing many taxa new to science. This overview explores this remarkable diversity and its potential to lead to new perspectives on the diversity and evolution of green plants. Molecular systematic and physiological data gathered from desert taxa demonstrate that these algae are long-term members of desert communities, not transient visitors from aquatic habitats. Variations in desiccation tolerance and photophysiology among these algae include diverse evolutionary innovations that developed under selective pressures in the desert. Combined with the single embryophyte lineage to which more familiar terrestrial green plants belong, multiple desert green algal lineages provide independent evolutionary units that may enhance understanding of the evolution and ecology of eukaryotic photosynthetic life on land.

Keywords: biodiversity, Chlorophyta, desert microbiotic crust, desiccation, green algae

Microbiotic crusts cover wide-ranging portions of the arid and semiarid regions of the world, keeping wind and water erosion in check, affecting water infiltration and runoff, influencing the establishment of vascular plants, and serving as major primary producers and nitrogen-fixing communities in arid landscapes (Belnap and Lange 2001). Although the crusts are extensive, they are also delicate, requiring decades to a millennium to reestablish after disturbances such as livestock trampling or vehicular traffic (Bowker 2007). Crusts comprise communities of cyanobacteria, lichens, bryophytes, green algae, diatoms, and other taxa that bind the upper layer of soil, producing a physical aggregation ranging in thickness from a few millimeters to several centimeters (figure 1; West 1990, St. Clair and Johansen 1993). The widespread distribution and varieties of crust communities, their fragility, and the recent desire to conserve and restore crusts in arid and semiarid lands have motivated extensive investigations of the physical and physiological characteristics of crusts and crust organisms and of the biodiversity of the crust community (Belnap and Lange 2001).

Recent reviews have summarized features of crust structure and function (West 1990, Evans and Johansen 1999, Belnap and Lange 2001); we focus here on two intriguing themes that are emerging as the hidden diversity of free-living desert green algae is revealed. First, the remarkable diversity hidden among the microscopic, unicellular desert green algae, spanning five distinct green algal classes and encompassing many previously unsampled taxa, provides a new perspective on the diversity and evolution of green plants. Second, the multiple, evolutionarily independent transitions from aquatic to desert habitats that we have detected among desert green algae reveal a gold mine of rich, natural variation that has evolved in the presence of strong selective pressures in the desert environment. All embryophytes (bryophytes, ferns, gymnosperms, and angiosperms) derive from a single algal lineage that transitioned from freshwater to dry land (Graham 1993). Hand in hand with the single embryophyte lineage, multiple desert green algal lineages provide independent evolutionary units for

the study of mechanisms that met the environmental challenges confronting the ancestor of embryophytes when it first made the leap from water to land. Such potential tolerance or avoidance mechanisms can be tested in an explicitly phylogenetic context, separating lineage-specific from habitat-specific traits. In this overview, we expand on these two major emerging themes.

Hidden diversity

At first glance, desert and other terrestrial green algae may seem to be a fairly narrow group of organisms. These microscopic, mostly unicellular eukaryotes range in diameter from 10 to 50 micrometers. Morphologically, they are spherical or rod-shaped in the vegetative stage and live singly or in small packets of cells (figure 2). Terrestrial green algae are often

Figure 1. Microbiotic crust habitats. (a) Gypsiferous crust community northwest of Albuquerque, New Mexico. Note the footpath worn in the thick crust layer, revealing the white gypsum beneath. (b) Crust community in southern Arizona. Photographs: Paul O. Lewis.

difficult to identify—examination of the nonmotile vegetative cells of different ages, the modes of cell division and reproduction, and alternate life stages (zoospores and gametes) that are motile by two or more cilia requires that they be isolated and cultured. Even though terrestrial green algae are rather simple morphologically, it is now evident that they are evolutionarily diverse, being found in six of ten green algal classes (figure 3). The surprising amount of diverse green algae in deserts parallels the recently uncovered diversity among eukaryotic microorganisms from habitats such as anoxic mud, highly acidic rivers, and deep-sea vents (e.g., Zettler et al. 2002).

Perspectives on the diversity of desert green algae have shifted dramatically over time with changes in the classification of green algae and with the use of data at increasingly finer levels of resolution. Traditional classification systems of green algae were based on vegetative cell morphology (e.g., Smith 1950); accordingly, classes and orders of green algae whose species had similar vegetative morphology were classified together. Later, specialists of unicellular green algae determined that vegetative cell features visible with light microscopy are less predictive of evolutionary relationships than internal cellular features, because the internal traits cut across diverse morphological forms. As electron microscopic data of algal cell division and motile cell structure (such as the swimming apparatus) became available for a large number of species, the green algae were divided into five classes based more on cell structure than on vegetative morphology (Mattox and Stewart 1984). A dramatic difference between the traditional system and the newer five-class system is that algae in one of the classes share motile cell features with those of land plants (e.g., sperm in bryophytes); this class is thus interpreted as having a closer evolutionary history to land plants (Stewart and Mattox 1978).

The five-class system has been developed further with the inclusion of DNA sequence data and an emphasis on phylogenetic systematics (i.e., naming monophyletic lineages). To resolve these deepest branches in the green plant tree of life, researchers have used data from slowly evolving regions in the nuclear 18S rDNA gene and the plastid *rbcL* (ribulose-biphosphate carboxylase) gene. At present, 10 classes of green algae have been named (figure 3). Additional classes are certain to be formalized as data are obtained from poorly studied groups such as the prasinophyte algae, which are recognized as a heterogeneous group of motile unicellular forms. Beyond providing a more complete picture of evolutionary relationships among different major groups of green algae, molecular phylogenetic studies, some including electron microscopy of motile stages, have demonstrated that many of the commonly encountered terrestrial genera (e.g., *Chlorella* and *Chlorococcum*) are not monophyletic (Nakayama et al. 1996, Huss et al. 1999). Taken as a whole, these findings indicate that species assessments based on morphological information from vegetative cells alone could underestimate green algal diversity, perhaps severely.
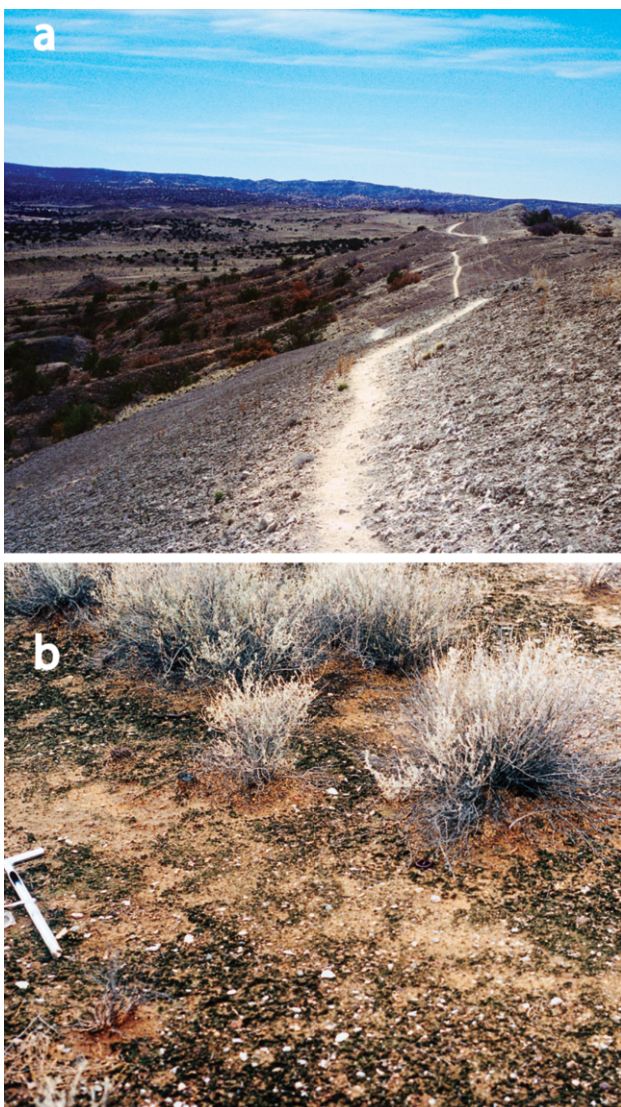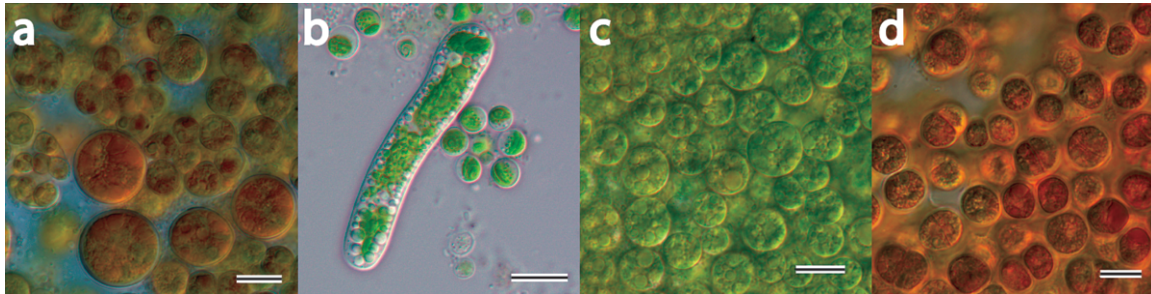
Figure 2. Examples of the green algae isolated from desert habitats. (a) **Scenedesmus rotundus** *(Chlorophyceae); (b)* **Cylindrocystis** *sp. (Zygnematophyceae); (c)* **Myrmecia** *sp. (Trebouxiophyceae); and (d)* **Chlorosarcinopsis** *sp. (Chlorophyceae). Scale bar = 10 micrometers. Photographs: Louise A. Lewis.*

Early taxonomic surveys of desert green algae employed only information about vegetative cell morphology (e.g., Friedmann et al. 1967, Metting 1981), resulting in a small number of species being described from desert habitats. Additional information from alternate life stages allowed phycologists to recognize many more desert species than had previously been described. For example, Flechtner and colleagues (1998) recovered 37 green algal taxa, representing 19 genera, from one location in Baja California, Mexico. As soil algae biologists who were assessing biodiversity (Flechtner 1999, Smith et al. 2004) pointed out, many of the morphologically similar taxa that have been recovered from distinct habitats are likely to represent distinct species. More recently, molecular phylogenetics has been used to make more accurate assessments of systematic relationships of desert green algae, and estimates of the number of different green algae that colonize desert habitats have risen (Lewis and Flechtner 2002, 2004), but sampling is still far from thorough.

To date, more than 400 strains of unicellular green algae have been cultured from a limited number of soil samples taken from arid habitats in the Sonoran, Mojave, Chihuahuan, and Great Basin regions of North America. These algae are currently held in the Biotic Crust Project collection (*http://hydrodictyon.eeb.uconn.edu/bcp/*). With 18S rDNA sequence data and information from other gene regions, we are using phylogenetic analyses to answer basic questions about the diversity of green algae of desert soils, such as: How many transitions to the desert habitat did green algae make? Is there evidence that green algae diversified in deserts, or did the algae isolated from desert soils develop from spores or other resistant structures recently dispersed from aquatic habitats?

Two papers illustrate the independent evolution of desert green algae from aquatic, freshwater green algae. With a small number (11) of green algal isolates from North American deserts, Lewis and Flechtner (2002) determined that desert green algae are not monophyletic, but instead arose within different classes of algae, and from freshwater ancestors. With a more extensive phylogenetic analysis of 18S rDNA sequence data from 23 desert and 127 nondesert green algae, Lewis and Lewis (2005) confirmed the freshwater

ancestry of desert green algae, and demonstrated that desert algae include members of five classes of green algae. These investigators identified multiple independent lineages of desert green algae within some of the classes. In Lewis and Lewis's (2005) study, the sampling of species included only isolates with known habitat data, so that the origins of all sequences on the tree could be designated as desert or nondesert habitat. Two methods were used to estimate the number of independent transitions to the desert habitat in green algae: with phylogenetic trees obtained from Bayesian phylogenetic analyses, parsimony reconstruction (under optimizations
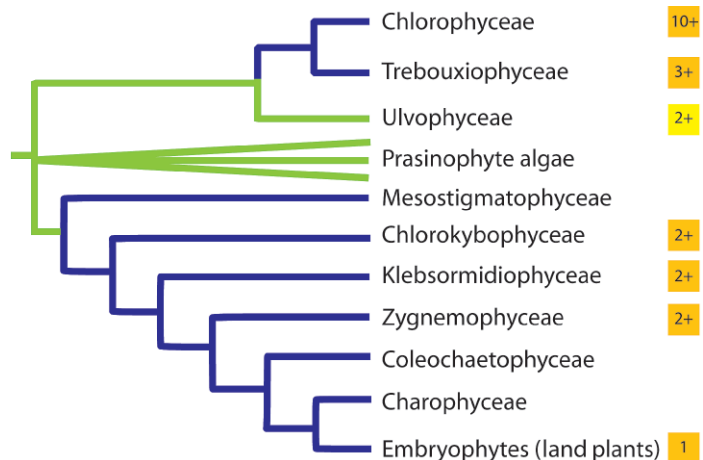
Figure 3. Summary phylogenetic tree of green plants from the green algal perspective. Shown are 10 classes of green algae and the single monophyletic lineage representing all embryophyte plants (bryophytes, vascular plants, seed plants, flowering plants). Branches on the tree are shaded green to indicate marine lineages and blue to denote freshwater lineages. Orange boxes indicate lineages containing desert biotic crust members, and the numbers in the boxes are conservative estimates of the number of independent terrestrial groups in each. The yellow box associated with Ulvophyceae represents lineages of marine-derived subaerial algae. Relationships and distribution of terrestrial taxa are summarized from Lewis and Flechtner (2004), Lewis and McCourt (2004), Smith and colleagues (2004), Lewis and Lewis (2005), López-Bautista and colleagues (2006), and Rindi and colleagues (2006).

favoring either reversals or parallel changes) and Bayesian mapping led to a conservative estimate of 14 to 17 independent transitions from aquatic ancestors to the desert habitat. This work did not take into account all of the sequences now in hand, however, and the number of desert lineages that we have detected is increasing with additional sampling. Interestingly, reversals from desert to aquatic habitats were not observed, but they may emerge as more isolates are analyzed.

In addition to being diverse phylogenetically, desert green algae also hold substantial DNA sequence variation that is not represented in public databases by green algae sampled from aquatic environments (Lewis and Lewis 2005). Comparisons of desert algae sequences with published sequences of aquatic algae from the National Center for Biotechnology Information (determined by BLAST [Basic Local Alignment Search Tool] analysis), along with analyses of desert and aquatic taxa on phylogenetic trees, indicate that some of the desert isolates are distantly related to known aquatic algae, whereas others are closely related to, and even nested within, known aquatic genera. If all of the desert taxa were closely related to already-sampled algae, they would represent only minor tip branches on the phylogenetic tree, and adding another sequence from a desert alga would increase the tree length only slightly. We observed, however, that including new data from desert algae adds significantly to the understanding of algal diversity as measured by the increase in total phylogenetic tree length (Lewis and Lewis 2005), which indicates that at least some of these algae are very distinct molecularly from known aquatic algae.

Phylogenetic analysis of DNA sequence data has generated important insights into the evolution and diversity of green algae from deserts, but it is becoming clear that 18S rDNA data provide only coarse-grained phylogenetic resolution, and they are not variable enough for assessing species-level questions. In some cases, cells with similar phenotypes and similar 18S rDNA sequences are found in both desert and aquatic habitats. Should these isolates be interpreted as a single species with wide ecological tolerances, or are they instead distinct taxa with similar morphology?

Lewis and Flechtner (2004) examined evidence for distinct species in cases where the 18S data indicated a close relationship to known aquatic green algae. They obtained six isolates from deserts in western North America. These very small cells resembled unicellular forms of the freshwater species *Scenedesmus obliquus.* At the level of 18S rDNA sequence similarity, the six desert isolates shared more than 99.6% similarity to the *S. obliquus* isolates from freshwater habitats in Sweden. For most purposes, they would be considered identical to the aquatic isolates. However, in phylogenetic analysis of a more variable region of the nuclear genome, the internal transcribed spacer (ITS) of rDNA, the desert isolates formed two well-supported clades, each possessing ITS haplotypes that were distinct from each other and from the aquatic *Scenedesmus* isolates. Using scanning electron microscopy, morphological distinctions were also found in the cell-wall surfaces. Thus, eukaryotic algae found in such different habitats as freshwater lakes and desert soils can have nearly identical morphology and 18S rDNA sequences, but possess variation in ITS rDNA that reveals evolutionary divergence between them.

The use of more variable molecular markers, here ITS rDNA, has provided a more complete picture of the diversity of green algae in desert soils and a better understanding of how quickly physiological differences can evolve. Data from studies using such markers, coupled with physiological contrasts discussed below, support the notion that desert green algae are not temporary visitors that recently dispersed from aquatic habitats. This information has important implications for improving the accuracy of biodiversity assessments and for enhancing understanding of the distribution of species in various habitats.

An obvious cautionary note about using cultured material to estimate desert algal diversity is that some (or even many) of the algae in the sampled desert crusts may have been missed because they are not easily cultured. Hawkes and Flechtner (2002) compared the algae detected from culturing with those detected from observing soils directly, and found 30% more species in the samples processed with direct observation. Other groups have also explored the diversity of prokaryotic crust microorganisms using the method of environmental sampling. Garcia-Pichel and colleagues (2001) compared microscopic and molecular assessments of cyanobacterial species in crusts and concluded that each method underestimated certain forms. We have noted on several occasions that the cell walls of the desert green algae we have in hand are very difficult to break open for access to DNA. It may be that very specialized techniques will have to be developed to capture DNA from all microorganisms present in crusts. Until then, studies using cultured algae will continue to elucidate the phylogenetic diversity of green algae in desert soils. In addition, physiological studies of cultured algae provide an opportunity to compare the biology of individual species of algae in desert soils with that of their closest aquatic relatives.

Physiology within the phylogenetic framework

The largest, most conspicuous, and best studied monophyletic group of green plants that has transitioned from water to land is the embryophytes, which includes the bryophytes and the vascular seedless, seeded, and flowering plants. The green plant group, however, also includes green algae, and it is clear from the work cited above that transitions by these unicellular green plants from freshwater to terrestrial habitats, even harsh deserts, have occurred multiple times. These dramatic habitat transitions, taking place in multiple, evolutionarily independent lineages, make up a diverse phylogenetic backdrop against which to examine physiological mechanisms and intracellular characteristics essential for green plant life on land.

In this context, a shift in perspective toward a more inclusive view of the suite of green plant physiologies could reveal drivers behind variation in chloroplast structure and function.

On the one hand, there is remarkable diversity across green algal taxa in such basic photophysiological characters as the presence and extent of thylakoid membrane stacking in grana within chloroplasts, separation of photosystems I and II within thylakoids, and carbon dioxide–concentrating mechanisms (Badger et al. 1998, Gunning and Schwartz 1999, Giordano et al. 2005). Though presence and morphology of grana are variable among green algal groups, chloroplast grana are ubiquitous in higher plants, so the higher plant condition could be viewed as one of very limited variation in a vast sea of variation on the evolutionary themes of green plant chloroplast form and function. On the other hand, variation in other photophysiological characteristics may be quite limited among green algal lineages, including, for example, in the common, distinctive, humped shape of the chlorophyll fluorescence induction curve during the first several hundred milliseconds of a saturating light pulse (Schreiber et al. 2002, Gray et al. 2006). The contrasting pattern in higher plants (in which chlorophyll fluorescence does not decline over the same time frame) could be considered unusual when compared with the green algal pattern in a taxonomically highly diverse framework. Why have different structures or behaviors appeared in embryophytes? Is this a lineage-specific fluke? Natural selection associated with the terrestrial habit? Are there common structural or physiological characters that unify terrestrial green photosynthetic organisms (algal and plant) and distinguish them from aquatic green algae?

An important first step toward using desert green algal lineages to begin exploring such questions is to ascertain whether the green algae in deserts became established and diverged there over generations, relative to their aquatic ancestors. Molecular evidence (outlined above; Lewis and Lewis 2005) suggests that indeed the desert green algae are not transient visitors from aquatic environments; their molecular sequences have diverged sufficiently from those of closely related, aquatic sister taxa to suggest that the desert taxa are long-term inhabitants of the desert. Recent parallel work by Gray and colleagues (2007) examining desiccation tolerance also has pointed clearly to distinct physiologies exhibited by desert and aquatic taxa in "common garden" experiments. One of the great hazards of desert life for all unicellular organisms is desiccation. For photosynthetic microalgae, desiccation threatens basic metabolism and can also heighten photodamage to the photosynthetic apparatus (e.g., Eickmeier et al. 1992, Casper et al. 1993, Baker and Bowyer 1994). There is an extensive body of literature exploring desiccation tolerance and photosynthetic response to desiccation and rehydration in diverse photosynthetic organisms, including beach-rock microbial mats (with green algae and cyanobacteria; Schreiber et al. 2002), lycophyte "resurrection plants" such as *Selaginella* species and some angiosperms (e.g., Eickmeier et al. 1992, Casper et al. 1993, Oliver et al. 2000, Rascio and La Rocca 2005), Antarctic mosses (e.g., Wasley et al. 2006), and cyanolichens and chlorolichens (e.g., Nash et al. 1990, Lange et al. 1997, Kopecky et al. 2005). These organisms possess a variety of protective mechanisms that, for example, dissi-

pate excess excitation energy when desiccation limits photosynthetic activity (e.g., Kopecky et al. 2005), or maintain the integrity of membranes through high concentrations of carbohydrates or lipids (e.g., Wasley et al. 2006).

Using phylogenetically diverse pairs of closely related desert and aquatic green algae from the Chlorophyceae and Trebouxiophyceae ($N = 6$ for desert taxa, $N = 5$ for aquatic taxa), Gray and colleagues (2007) used phylogenetically informed statistical methods to explore the ability of desert algae and close sister aquatic taxa to reinitiate photosynthesis after exposure to short- and long-term desiccation stress. Desert and aquatic algae were grown in unialgal culture on porous glass beads contained in small, mesh-bottomed acrylic containers (figure 4, inset). Moisture in these small containers could be controlled by placing them in larger petri plates and fully hydrating them by allowing water to wick through the mesh bottom, or by controlling humidity in the air flowing through the petri plate container. Algae were desiccated either in the dark or under day–night illumination cycles, and photosynthetic quantum yield (ϕ_{PSII}) was observed from dark-adapted samples using chlorophyll fluorimetry. The quantum yield for all algae declined to near 0 (indicating loss of photosynthetic activity) after 12 to 24 hours of exposure to a drying (25% humidity) airstream. Algae were then allowed to remain dry for an additional 24 hours, 7 days, or 4 weeks in the growth room, after which they were rehydrated and assayed for recovery of ϕ_{PSII}. Dark-adapted chlorophyll fluorescence was measured at 1 hour, 24 hours, and 48 hours into rehydration in darkness, and again following reillumination for 24 hours and for 5 days in the growth chamber.

Overall, isolates from desert crusts maintained quite high ϕ_{PSII} after 24 hours and after 7 days of desiccated conditions, particularly when they were desiccated in the dark. Even after 4 weeks of desiccation, ϕ_{PSII} was not 0 when desert algae desiccated in the dark were rehydrated for just one hour (figure 4). This ability to resurrect photosynthesis was not associated with obvious morphological changes, which would have indicated entry into spore stages during desiccation. In contrast, aquatic taxa overall exhibited greatly reduced ϕ_{PSII} relative to the baseline hydrated condition, even after enduring only 24 hours of full desiccation (Gray et al. 2007). After 4 weeks of desiccation in light or darkness, ϕ_{PSII} recovery in aquatic algae rehydrated in darkness was very low, though it could rebound after longer-term reillumination of the rehydrated samples (figure 4). The large error bars associated with ϕ_{PSII} in aquatic algae (figure 4) do not indicate that data from single species were variable; instead, there was dramatic variation among aquatic species in their capacity to recover ϕ_{PSII} after desiccation (for details, see Gray et al. 2007). Algae isolated from desert crusts clearly have superior tolerance for desiccation; however, desert algae desiccated under diel light–dark regimes (rather than continuously in the dark) sustained greater damage and more strongly reduced ϕ_{PSII} than dark-desiccated taxa. The mechanisms associated with these differences in tolerance of photosynthesis to
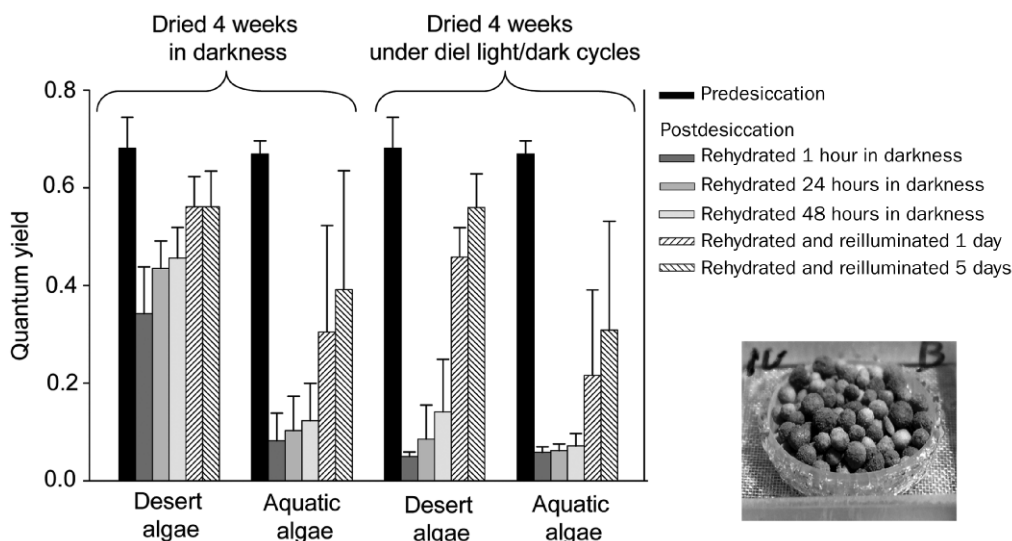
Figure 4. Quantum yield of photosystem II determined from chlorophyll fluorescence before and after desiccation of algae, measured from desert and aquatic algae established in separate, unialgal cultures on porous glass beads (inset photograph). Error bars represent the standard error for each group. Photograph: Dennis W. Gray.

the well-studied *Tortula,* can dry quickly (in less than an hour) and return to normal photosynthetic yield within two hours after rehydration. Bryophytes use constitutive expression of protective components, such as dehydrin proteins and sugars, as well as rapid repair mechanisms (Oliver et al. 2000, 2005).

The desert green algae we have examined exhibit dynamics of desiccation tolerance similar to those of mosses, in that vegetative tissues can tolerate rapid dehydration, and cellular functions such as photosynthesis can recover upon rehydration very quickly (Gray et al. 2007).

desiccation and illumination are unknown and under continuing investigation.

Beyond this photosynthetic tolerance to desiccation, general mechanisms of cellular desiccation tolerance have yet to be examined in desert algae; these mechanisms protect the photosynthetic apparatus and maintain cellular integrity during desiccation and upon rehydration. It is clear that green algae have "resting" stages (spores and zygotes) that allow cells to overwinter in freezing temperatures or to survive from year to year in ephemeral pools. Trainor and Gladych (1995) found that even after soils had air-dried for 35 years, green algae—which had survived in unknown form—could be cultured from them. In the physiological experiments described above, however, the green algae survive desiccation in the vegetative state.

Vegetative desiccation tolerance is widespread in embryophytes, appearing in bryophytes, ferns, and many angiosperms, but tolerant species are rare (Alpert 2000). Oliver and colleagues (2000, 2005) hypothesized that vegetative desiccation tolerance may be the ancestral condition, often associated with the ability to tolerate and quickly rebound from rapid dehydration, as in bryophytes. Desiccation tolerance in the diploid (sporophyte) vegetative tissues of angiosperms and ferns, in contrast, can be induced when drying is slow, and recovery upon rehydration can be sluggish (Oliver et al. 2000). For example, in an overview of strategies and dynamics of desiccation tolerance and recovery in an angiosperm, a lichen, and a moss, Tuba and colleagues (1998) noted that up to 72 hours are required postrehydration for photosynthetic recovery in the desiccation-tolerant angiosperm *Xerophyta scabrida.* By comparison, desiccation-tolerant haploid (gametophyte) cells of desert bryophytes, such as

In some cases, more than 90% of initial photosynthetic yield was recovered within one hour after rewetting of vegetative tissues of desert algae desiccated in darkness. At this point we do not know if the green algae express dehydrin-like proteins or use repair mechanisms seen in desert mosses, but, as Oliver and colleagues (2005) discussed, a key to understanding the evolution of widespread vegetative desiccation tolerance in bryophytes is knowledge of protective and repair mechanisms in green algae that share a common ancestor with embryophyte land plants. Examining desiccation tolerance mechanisms in multiple, independent lineages of desert green algae may deepen knowledge of ancestral conditions of vegetative desiccation tolerance in green photosynthetic organisms in general.

Given that both molecular and physiological data indicate that desert green algae are not just transient aquatic visitors in the desert landscape, these desert green algal lineages provide potentially very powerful, independent evolutionary units with which to examine lineage-specific and habitat-specific patterns in photosynthetic structure and function. One basic process for photosynthetic organisms that lends itself to analysis in this phylogenetic context is energy sharing among photosystems I and II. In higher plants, photosystem II (PSII) is concentrated in appressed regions of granal stacks of thylakoid membranes, and photosystem I (PSI) is located in thylakoids between grana (Stys 1995, Staehelin 2003, Dekker and Boekema 2005). Anderson (1999, 2002) hypothesized that this separation of photosystems within thylakoid membranes may be advantageous, given light conditions in the terrestrial habitat. Light attenuation by terrestrial canopies tends to enrich the far-red wavelengths, which are preferentially absorbed by PSI, in lower canopy layers. The spatial segregation

of PSII and PSI in granal-appressed and nonappressed thylakoid regions, respectively, minimizes the excitation transfer from PSII antennae to PSI (termed spillover), as well as the preferential use of excitation energy by PSI driven simply by its rapid reaction rates (Trissl and Wilhelm 1993). Overall photosynthetic efficiency is enhanced. Anderson (1999, 2002) further hypothesized that the more homogeneous distributions of PSI and PSII, and less-developed grana, in chloroplasts of many common aquatic green algae may improve photosynthetic function in water, where far-red wavelengths are the first wavelengths lost with depth. Attenuated light in deeper water is enriched with light preferentially absorbed by PSII, and the mixing of PSII and PSI in algal thylakoids may enhance excitation spillover from PSII to PSI, improving overall photosynthetic efficiency under water.

The implicit idea is that grana stacking may correlate with green plant life on land, and a lack of stacking may correlate with green plant life in water. Closely related desert and aquatic green algae provide the opportunity to test this idea. Embryophytes exhibit one strategy for dealing with light quality "skewed" by the terrestrial habit—the separation of the photosystems associated with grana stacking of thylakoid membranes. Other examples of successful strategies may lie in the multiple lineages of desert green algae. The presence of grana may, in fact, be lineage specific, not linked to habitat.

In a survey of aquatic green algae, Gunning and Schwartz (1999) used confocal microscopy to examine patterns of chlorophyll fluorescence emitted from chloroplasts of intact green algal cells. Punctate, bright fluorescence was assumed to indicate the presence of grana stacks in which PSII was concentrated, as is true in higher plants. Their work showed that the extent of grana development depended on green algal lineage, at least within the limits of the taxa sampled. In particular, algae in Zygnemophyceae, Coleochaetophyceae, and Charophyceae appeared to have grana, whereas algae from other classes (with the exception of *Cladophora*, Ulvophyceae) tended not to exhibit clear grana stacking. Gunning and Schwartz (1999) specifically noted that surveys of *Klebsormidium, Mesostigma,* and *Chlorokybus* genera in classes that diverged even earlier than did Zygnemophyceae are essential for exploring the distribution of the grana-stacking character within the green algal phylogeny; *Klebsormidium* is well represented within desert algal isolates. Whether all members of these classes have well-developed grana remains unclear; in particular, do desert algae in these classes have well-developed grana, arguably for similar reasons that terrestrial plants do? Using transmission electron microscopy, McManus and colleagues (2005) clearly showed that an aquatic species of Zygnemophyceae exhibits extensive stacking of the thylakoid membranes in grana, yet observation of similarly extensive stacking in a phylogenetically close desert relative has remained elusive. The potential implications are striking; differential extents of stacking in grana among lineages of desert green algae could drive substantially different mechanisms for sharing of excitation energy between PSI and

PSII, potentially with ramifications for handling of excitation energy by the xanthophyll cycle (or its equivalent; e.g., Niyogi et al. 1997, Niyogi 1999) and by state transitions (Finazzi et al. 2001, Wollman 2001).

Interestingly, Mullineaux (2005) suggests that the separation of photosystems is solely a side effect of granal stacking driven by a completely different impetus—the need for a larger light-harvesting antenna for PSII under terrestrial shady conditions, coupled with diffusion constraints faced by plastoquinone. However, Mullineaux's accompanying suggestion, that the chloroplasts of algal precursors of land plants may have lost phycobilisomes in response to high light when living at the aquatic-terrestrial interface, does not correspond with the current understanding of green algal evolution. As detailed above, embryophytes are a comparatively recent branch of the green algal tree of life. Because all extant lineages of green algae, from prasinophytes to chlorophytes to charophytes, lack phycobilisomes, it is unlikely that phycobilisomes were lost during the transition to a terrestrial light environment. Molecular dating (Yoon et al. 2004) places the most recent common ancestor of all extant green algae at 1100 million to 1200 million years ago, far earlier than the origin of embryophytes at 420 million to 430 million years ago (Kenrick and Crane 1997).

In sum, crusts are a natural, ongoing laboratory featuring unrelated aquatic taxa that diversified to the desert habit. Ultimately, these evolutionary "experiments" could provide very interesting information about protection against the effects of extreme dehydration, a threat to all known forms of life, and photoprotection under environmental stress, a threat to major primary producers. As in most ecosystems on Earth, the soil microbial community is a frontier, and our understanding of how such communities evolve, influence, and even create their own environment by constructing complex assemblages of various thicknesses, textures, and biochemistries is essential for understanding their basic influences on the biogeochemistry of Earth's atmosphere and ecosystems.

Learning more about the cryptic diversity of desert green algae and their ecological tolerances and physiology also has practical application. The green algae are among the major structural and photosynthetic constituents of desert crusts, along with cyanobacteria (often dominant), bryophytes, lichens, and fungi, and the physiology of these microscopic, single-celled green algae distributed within microbiotic crusts is practically impossible to study *in situ.* Yet in cooler habitats that are slightly more moist or acidic, green algae can be the dominant organisms in microbiotic crusts (Büdel 2001, Johansen 2001, Smith et al. 2004). As efforts to restore crusts intensify (Bowker 2007), knowledge of preferences, physiology, tolerances, and sensitivities of dominant desert crust organisms, including green algae, will be essential information.

Crusts are targets for restoration for several ecological reasons. They can be dominant sources of productivity and carbon sequestration in extremely dry environments, and they can contribute to soil fertility through the fixation of

nitrogen (Evans and Johansen 1999). Land managers have long noted the ability of crusts to bind and stabilize soils, preventing erosion (Belnap and Lange 2001). And the diverse physical structures of crusts change water movement over soil surfaces, in some cases enhancing water penetration and providing favorable microsites for the germination of seeds, in others promoting sheeting of water flow over the landscape. Unfortunately, biotic crusts are also very fragile structures that are susceptible to physical disturbances such as livestock trampling and vehicular traffic, and they recover only very slowly (Belnap and Lange 2001, Bowker 2007). Interest in organismal diversity in crusts, crust function, crust establishment, and crust management has thus grown greatly among managers of western arid and semiarid lands as sustainability and productivity become interlinked goals.

Acknowledgments

References cited

Alpert P. 2000. The discovery, scope, and puzzle of desiccation tolerance in plants. Plant Ecology 151: 5–17.

Anderson JM. 1999. Insights into the consequences of grana stacking of thylakoid membranes in vascular plants: A personal perspective. Australian Journal of Plant Physiology 26: 625–639.

———. 2002. Changing concepts about the distribution of photosystems I and II between grana-appressed and stroma-exposed thylakoid membranes. Photosynthesis Research 73: 157–164.

Badger MR, Andrews TJ, Whitney SM, Ludwig M, Yellowlees DC, Leggat W, Price GD. 1998. The diversity and coevolution of Rubisco, plastids, pyrenoids, and chloroplast-based CO_2-concentrating mechanisms in algae. Canadian Journal of Botany 76: 1052–1071.

Baker NR, Bowyer JR, eds. 1994. Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field. Oxford (United Kingdom): BIOS Scientific.

Belnap J, Lange OL, eds. 2001. Biological Soil Crusts: Structure, Function, and Management. Heidelberg (Germany): Springer.

Bowker MA. 2007. Biological soil crust rehabilitation in theory and practice: An underexploited opportunity. Restoration Ecology 15: 13–23.

Büdel B. 2001. Synopsis: Comparative biogeography of soil-crust biota. Pages 141–152 in Belnap J, Lange OL, eds. Biological Soil Crusts: Structure, Function, and Management. Heidelberg (Germany): Springer.

Casper C, Eickmeier WG, Osmond CB. 1993. Changes of fluorescence and xanthophyll pigments during dehydration in the resurrection plant *Selaginella lepidophylla* in low and medium light intensities. Oecologia 94: 528–533.

Dekker JP, Boekema EJ. 2005. Supramolecular organization of thylakoid membrane proteins in green plants. Biochimica et Biophysica Acta—Bioenergetics 1706: 12–39.

Eickmeier WG, Lebkuecher JG, Osmond CB. 1992. Photosynthetic water oxidation and water stress in plants. Pages 223–239 in Somero GN, Osmond CB, Bolis CL, eds. Water and Life. Berlin: Springer.

Evans RD, Johansen JR. 1999. Microbiotic crusts and ecosystem processes. Critical Reviews in Plant Sciences 18: 183–225.

Finazzi G, Barbagallo RP, Bergo E, Barbato R, Forti G. 2001. Photoinhibition of *Chlamydomonas reinhardtii* in state 1 and state 2: Damages to the photosynthetic apparatus under linear and cyclic electron flow. Journal of Biological Chemistry 276: 22251–22257.

Flechtner VR. 1999. Enigmatic desert soil algae: Soil algal flora of the western U.S.A. and Baja California, Mexico. Pages 233–241 in Seckbach J, ed. Enigmatic Microorganisms and Life in Extreme Environments. Dordrecht (Netherlands): Kluwer Academic.

Flechtner VR, Johansen JR, Clark WH. 1998. Algal composition of microbiotic crusts from the central desert of Baja California, Mexico. Great Basin Naturalist 58: 295–311.

Friedmann EI, Lipkin Y, Ocampo-Paus R. 1967. Desert algae of the Negev (Israel). Phycologia 7: 185–200.

Garcia-Pichel F, López-Cortés A, Nübel U. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. Applied and Environmental Microbiology 67: 1902–1910.

Giordano M, Beardall J, Raven JA. 2005. CO_2 concentrating mechanisms in algae: Mechanisms, environmental modulation, and evolution. Annual Review of Plant Biology 56: 99–131.

Graham LE. 1993. Origin of Land Plants. New York: Wiley.

Gray DW, Cardon ZG, Lewis LA. 2006. Simultaneous collection of rapid chlorophyll fluorescence induction kinetics, fluorescence quenching parameters, and environmental data using an automated PAM-2000/CR10x data logging system. Photosynthesis Research 87: 295–301.

Gray DW, Lewis LA, Cardon ZG. 2007. Photosynthetic recovery following desiccation of desert green algae (Chlorophyta) and their aquatic relatives. Plant, Cell, and Environment 30: 1240–1255.

Gunning BES, Schwartz OM. 1999. Confocal microscopy of thylakoid autofluorescence in relation to origin of grana and phylogeny in the green algae. Australian Journal of Plant Physiology 26: 695–708.

Hawkes CV, Flechtner VR. 2002. Biological soil crusts in a xeric Florida shrubland: Composition, abundance, and spatial heterogeneity of crusts with different disturbance histories. Microbial Ecology 43: 1–12.

Huss VAR, Frank C, Hartmann EC, Hirmer M, Kloboucek A, Seidel BM, Wenzeler P, Kessler E. 1999. Biochemical taxonomy and molecular phylogeny of the genus *Chlorella* sensu lato (Chlorophyta). Journal of Phycology 35: 587–598.

Johansen JR. 2001. Impacts of fire on biological soil crusts. Pages 385–397 in Belnap J, Lange OL, eds. Biological Soil Crusts: Structure, Function, and Management. Heidelberg (Germany): Springer.

Kenrick P, Crane PR. 1997. The origin and early evolution of plants on land. Nature 389: 33–39.

Kopecky J, Azarkovich M, Pfündel EE, Shuvalov VA, Heber U. 2005. Thermal dissipation of light energy is regulated differently and by different mechanisms in lichens and higher plants. Plant Biology 7: 156–167.

Lange OL, Belnap J, Reichenberger H, Meyer A. 1997. Photosynthesis of green algal soil crust lichens from arid lands in southern Utah, USA: Role of water content on light and temperature responses of CO_2 exchange. Flora 192: 1–15.

Lewis LA, Flechtner VR. 2002. Green algae (Chlorophyta) of desert microbiotic crusts: Diversity of North American taxa. Taxon 51: 443–451.

———. 2004. Cryptic species of *Scenedesmus* (Chlorophyta) from desert soil communities of western North America. Journal of Phycology 40: 1127–1137.

Lewis LA, Lewis PO. 2005. Unearthing the molecular phylodiversity of desert soil green algae (Chlorophyta). Systematic Biology 54: 936–947.

Lewis LA, McCourt RM. 2004. Green algae and the origin of land plants. American Journal of Botany 91: 1535–1556.

López-Bautista JM, Rindi F, Guiry MD. 2006. Molecular systematics of the subaerial green algal order Trentepohliales: An assessment based on morphological and molecular data. International Journal of Systematic and Evolutionary Microbiology 56: 1709–1715.

Mattox KR, Stewart KD. 1984. Classification of the green algae: A concept based on comparative cytology. Pages 29–72 in Irvine DEG, John DM, eds. The Systematics of the Green Algae. London: Academic Press.

McManus HA, Cardon ZG, Lewis LA. 2005. Does thylakoid stacking follow a phylogenetic pattern? Poster presented at the Phycological Society of America meeting; 13–19 August 2005, Durban, South Africa.

Metting B. 1981. The systematics and ecology of soil algae. Botanical Review 47: 195–312.

Mullineaux CW. 2005. Function and evolution of grana. Trends in Plant Science 10: 521–525.

Nakayama T, Watanabe S, Mitsui K, Uchida H, Inouye I. 1996. The phylogenetic relationship between the Chlamydomonadales and Chlorococcales inferred from 18S rDNA data. Phycological Research 44: 47–55.

Nash TH, Reiner A, Demmig-Adams B, Kilian E, Kaiser WM, Lange OL. 1990. The effect of atmospheric desiccation and osmotic water stress on photosynthesis and dark respiration of lichens. New Phytologist 116: 269–276.

Niyogi KK. 1999. Photoprotection revisited: Genetic and molecular approaches. Annual Review of Plant Physiology and Plant Molecular Biology 50: 333–359.

Niyogi KK, Bjorkman O, Grossman AR. 1997. The roles of specific xanthophylls in photoprotection. Proceedings of the National Academy of Sciences 94: 14162–14167.

Oliver MJ, Tuba Z, Mishler BC. 2000. The evolution of vegetative desiccation tolerance in land plants. Plant Ecology 151: 85–100.

Oliver MJ, Velten J, Mishler BC. 2005. Desiccation tolerance in bryophytes: A reflection of the primitive strategy for plant survival in dehydrating habitats? Integrative and Comparative Biology 45: 788–799.

Rascio N, La Rocca N. 2005. Resurrection plants: The puzzle of surviving extreme vegetative desiccation. Critical Reviews in Plant Sciences 24: 209–225.

Rindi F, López-Bautista J, Sherwood A, Guiry M. 2006. Morphology and phylogenetic position of *Spongiochrysis hawaiiensis* gen et sp. nov., the first known terrestrial member of the Cladophorales (Ulvophyceae, Chlorophyta). International Journal of Systematic and Evolutionary Microbiology 56: 913–922.

Schreiber U, Gademann R, Bird P, Ralph PJ, Larkum AWD, Kuhl M. 2002. Apparent light requirement for activation of photosynthesis upon rehydration of desiccated beachrock microbial mats. Journal of Phycology 38: 125–134.

Smith GM. 1950. The Freshwater Algae of the United States. New York: McGraw-Hill.

Smith SM, Abed RMM, Garcia-Pichel F. 2004. Biological soil crusts of sand dunes in Cape Cod National Seashore, Massachusetts, USA. Microbial Ecology 48: 200–208.

St. Clair LL, Johansen JR. 1993. Introduction to the symposium on soil crust communities. Great Basin Naturalist 53: 1–4.

Staehelin LA. 2003. Chloroplast structure: From chlorophyll granules to supra-molecular architecture of thylakoid membranes. Photosynthesis Research 76: 185–196.

Stewart KD, Mattox KR. 1978. Comparative evolution in the flagellated cells of green algae and land plants. BioSystems 10: 145–152.

Stys D. 1995. Stacking and separation of photosystem I and photosystem II in plant thylakoid membranes: A physico-chemical view. Physiologia Plantarum 95: 651–657.

Trainor FR, Gladych R. 1995. Survival of algae in a desiccated soil: A 35-year study. Phycologia 34: 191–192.

Trissl HW, Wilhelm C. 1993. Why do thylakoid membranes from higher plants form grana stacks? Trends in Biochemical Sciences 18: 415–419.

Tuba Z, Protor CF, Csintalan Z. 1998. Ecophysiological responses of homoiochlorophyllous and poikilochlorophyllous desiccation tolerant plants: A comparison and an ecological perspective. Plant Growth Regulation 24: 211–217.

Wasley J, Robinson SA, Lovelock CE, Popp M. 2006. Some like it wet—biological characteristics underpinning tolerance of extreme water stress events in Antarctic bryophytes. Functional Plant Biology 33: 443–455.

West NE. 1990. Structure and function of microphytic soil crusts in wild ecosystems of arid to semiarid regions. Advances in Ecological Research 20: 179–223.

Wollman FA. 2001. State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO Journal 20: 3623–3630.

Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. 2004. A molecular timeline for the origin of photosynthetic eukaryotes. Molecular Biology and Evolution 21: 809–818.

Zettler LA, Gomez F, Zettler E, Keenan BG, Amils R, Sogin ML. 2002. Eukaryotic diversity in Spain's River of Fire. Nature 417: 137.