Photosynthetic recovery following desiccation of desert green algae (Chlorophyta) and their aquatic relatives

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ABSTRACT

Recent molecular data suggest that desert green algae have evolved from freshwater ancestors at least 14 times in three major classes (Chlorophyceae, Trebouxiophyceae and Charophyceae), offering a unique opportunity to study the adaptation of photosynthetic organisms to life on land in a comparative phylogenetic framework. We examined the photorecovery of phylogenetically matched desert and aquatic algae after desiccation in darkness and under illumination. Desert algae survived desiccation for at least 4 weeks when dried in darkness, and recovered high levels of photosynthetic quantum yield within 1 h of rehydration in darkness. However, when 4 weeks of desiccation was accompanied by illumination, three of six desert taxa lost their ability to recover quantum yield during rehydration in the dark. Aquatic algae, in contrast, recovered very little during dark rehydration following even just 24 h of desiccation. Re-illuminating rehydrated algae produced a nearly complete recovery of quantum yield in all desert and two of five aquatic taxa. These contrasts provide physiological evidence that desert green algae possess mechanisms for photosynthetic recovery after desiccation distinct from those in aquatic relatives, corroborating molecular evidence that they are not happenstance, short-term visitors from aquatic relatives, but instead their DNA sequences have evolved sufficiently to be considered taxa distinct from aquatic sister taxa (Lewis & Flechtner 2002). Molecular data also indicate that these desert green algal isolates are not simply incidental visitors blown in, for example, on wind currents, but instead their DNA sequences have evolved sufficiently to be considered taxa clearly distinct from aquatic sister taxa (Lewis & Flechtner 2002). Whether these desert algae possess distinct physiological features that distinguish them from their aquatic counterparts is presently unknown.

Key-words: chlorophyll fluorescence; desert crust; light stress; photodamage; photoprotection.

INTRODUCTION

Living in a desert presents an enormous challenge for photosynthetic organisms. Deserts are dry, experience temperature extremes (hot and cold) and expose their inhabitants to intense solar radiation during periods of time when the potential for dissipating absorbed energy through photosynthetic activity is limited by temperature or the lack of water. Together these stresses make deserts some of the least favourable places for unicellular photosynthetic organisms to live. Yet, despite these stresses, many desert regions contain a tremendous diversity of microscopic life, much of which is organized into a biological crust at the soil surface.

Desert biological soil crusts are typically dominated by cyanobacteria, but may also contain bryophytes, lichens, fungi and can even be dominated by free-living unicellular green algae in moister, colder environments (Cameron 1960; Shields & Drouet 1962; Cameron 1964; Friedmann & Ocampo-Paus 1967; Belnap, Budel & Lange 2001). During periods of desiccation, many crust organisms lie dormant. However, when water becomes available, they can quickly become physiologically active (Rosentreter & Belnap 2001) and play important ecological roles as primary producers (Garcia-Pichel & Belnap 1996) and, in the case of the cyanobacteria, by fixing nitrogen (Evans & Ehleringer 1993; Belnap 2003).

Recent molecular studies have revealed surprising taxonomic diversity among unicellular green algae in biotic crusts, spread across three major green algal classes (sensu Mattox & Stewart 1984): Chlorophyceae, Trebouxiophyceae and Charophyceae (Lewis & Flechtner 2002). Molecular data also indicate that these desert green algal isolates are not simply incidental visitors blown in, for example, on wind currents, but instead their DNA sequences have evolved sufficiently to be considered taxa clearly distinct from aquatic sister taxa (Lewis & Flechtner 2002). Whether these desert algae possess distinct physiological features that distinguish them from their aquatic counterparts is presently unknown.

The eukaryotic green desert algae offer a unique opportunity for studying the adaptation of photosynthetic organisms to terrestrial life. The traditional approach to investigating the adaptation to land in photosynthetic organisms has involved comparisons of photosynthetic physiology in embryophytes (terrestrial green plants) and aquatic algae. However, embryophytes are descendants of a single evolutionary transition from an aquatic ancestor to terrestrial descendants, so only one statistically independent comparison is encapsulated even in comparisons of...
multiple land plants with multiple aquatic algae (Felsenstein 1985; Harvey & Pagel 1991). In contrast, the eukaryotic desert green algae represent multiple evolutionary lineages that have arisen independently from aquatic ancestors at least 14 times (Lewis & Lewis 2005). By comparing independently derived desert lineages with related aquatic algae, it is possible to have multiple statistically independent comparisons that are not possible when terrestrial plants are compared with algae. These evolutionarily independent transitions or replicated shifts to terrestrial life seen in the desert green algae (Lewis & Flechtner 2002; Lewis & Lewis 2005) facilitate the investigation of whether shifts in habitat (aquatic to desert) are correlated with changes in physiological traits in a statistically robust comparative phylogenetic framework.

In making the transition from an aquatic habitat to a terrestrial one (desert crusts), unicellular algae have had to cope with radically different ecological conditions. Compared to terrestrial environments, aquatic ones are relatively thermally stable (owing to the high heat capacity of water). Terrestrial environments, on the other hand, lack this thermal buffering and their inhabitants must be capable of tolerating changes (often rapid) in temperature. In aquatic environments, desiccation is rarely a problem, while in terrestrial environments, desiccating conditions are the norm. In terrestrial environments (especially deserts), these periods of desiccation are interspersed with brief periods of water availability following rain. Yet, it is important to recognize that some aquatic environments, such as intertidal regions and ephemeral ponds, may experience desiccation at regular intervals, blurring the distinction between aquatic and terrestrial habitats. Aquatic and terrestrial habitats also differ in the light environments their inhabitants are exposed to. Because light is attenuated more rapidly through a column of water than through a column of air, aquatic environments typically have lower light intensities than terrestrial ones. However, if an aquatic organism drifts freely in the water column, the light environment it experiences may be more variable than the one it would experience in a terrestrial environment where mobility is more limited, and organisms (algae in particular) are limited to residing on surfaces. Given the differing ecological conditions likely experienced by aquatic and terrestrial organisms, it would be surprising if they did not possess adaptations for their respective environments.

In this study, we attempt to better understand the physiological differences between desert and aquatic green algae, in hopes of gaining insight into the evolutionary transition to land in photosynthetic organisms. Specifically, we examined the response of multiple, independent lineages of desert green algae and their aquatic relatives to two environmental stresses: desiccation and illumination during desiccation. By analysing data from desert and aquatic isolates using a statistical comparative approach (Felsenstein 1985; Harvey & Pagel 1991; Garland, Bennett & Rezende 2005), any potentially confounding impacts of shared evolutionary history among algae were removed. We examined desiccation tolerance in the dark and in light, and the rapidity with which photosynthetic activity could recover after desiccation; these dynamics are a first step towards characterizing potential damage-tolerance and damage-avoidance mechanisms in these desert green algae based on mechanisms known in other photosynthetic organisms in the literature. For example, the cyanobacterium *Nostoc flagelliforme* recovers photosynthetic ability slowly following rehydration and appears to rely on repair processes requiring protein synthesis (Qiu et al. 2004), while recovery of photosynthetic activity in the cyanobacterium *Microcoleus* sp. occurs rapidly following rehydration and does not require de novo protein synthesis (Harel, Ohad & Kaplan 2004). Within bryophytes, some species recover full photosynthetic ability within minutes of rehydration of the haploid vegetative tissues (Marshall, Proctor & Smirnoff 1998; Csintalan, Proctor & Tuba 1999; Proctor & Smirnoff 2000) and tolerate very rapid drying rates (Oliver & Bewley 1997; Oliver, Tuba & Mishler 2000), suggesting the existence of strong, constitutively expressed protective mechanisms that prevent damage to their photosynthetic systems during desiccation. In contrast, other species recover more slowly and appear to require gradual drying to allow time for the synthesis of protective proteins such as ‘dehydrins’ (Oliver & Bewley 1997; Oliver et al. 2000). This latter pattern is also seen in the lycophyte ‘resurrection plant’ *Selaginella* (Schwab, Schreiber & Heber 1989; Muslin & Homann 1992; Oliver & Bewley 1997) and in certain angiosperms (Vicre, Farrant & Driouich 2004).

Very little is known about the adaptations allowing single-celled, eukaryotic green algae to survive in desert crusts. However, because these desert algae are morphologically very simple (Lewis & Flechtner 2004) and lack obvious structural features to distinguish them from their aquatic relatives, their abilities to survive and grow in the desert environment are likely the result of changes in physiology rather than persistent changes in gross morphology. During desiccation, membranes may become physically disrupted by the loss of water (Bewley 1979; Crowe, Hoekstra & Crowe 1992), and cells may suffer from both chemical and oxidative damage when dry (Smirnoff 1993; Weissman, Garty & Hochman 2005b). To cope with such potential sources of damage, photosynthetic organisms more generally have evolved an array of physiological protective mechanisms, including osmotic adjustments to the cytoplasm that reduce damage to membranes and organelles (Crowe et al. 1992; Smirnoff 1992; Muller et al. 1997; Potts 1999), synthesis of proteins that protect cellular components during the desiccation process (Gwodz, Bewley & Tucker 1974; Oliver 1996), and the production of antioxidants and scavenging enzymes that neutralize reactive oxygen species (ROS) generated during drying (Seel, Hendry & Lee 1992; Kraner et al. 2003; Ledford & Niyogi 2005; Weissman, Garty & Hochman 2005a). Carotenoids, especially those of the xanthophyll cycle, serve to harmlessly dissipate excess energy absorbed by chlorophyll in terrestrial plants and algae (Bilger & Bjorkman 1990; Demmig-Adams & Adams 1992; Gilmore 1997; Niyogi 1999; Bukhov et al. 2001a,b; Masojidek et al. 2004).
cyanobacteria, which lack a xanthophyll cycle, some species synthesize screening pigments that shield them from UV radiation (Bohm et al. 1995; Potts 1999). Finally, upon rehydration, desiccated organisms may repair damage by synthesizing new proteins to replace damaged ones (Gwozdz & Bewley 1975; Oliver & Bewley 1984a,b; Oliver 1996; Oliver & Bewley 1997; Oliver, Wood & O’Mahony 1997). Among some aquatic green algae (such as *Spirogyra* and *Volvox*), survival of desiccation is linked to switches in life stage; the diploid zygote can tolerate adverse environmental conditions and then undergo meiosis to produce active, haploid vegetative cells. However, such a pattern among desert green algae as a strategy for tolerating desiccation has not been generally documented. The degree to which photosynthetic organisms of all complexities rely on specific mechanisms to tolerate desiccation, and their relative reliance on protection versus repair strategies, clearly varies considerably (Bewley & Oliver 1992; Oliver & Bewley 1997). The potentially diverse mechanisms used by phylogenetically diverse desert green algae are virtually unknown but are of interest both for understanding the evolution of unicellular life on land and for restoration efforts in arid lands where crusts have been disrupted.

**MATERIALS AND METHODS**

**Study organisms**

In this study, we examine the physiology of 11 eukaryotic green algae that live in contrasting habitats (desert and aquatic) (Table 1). Desert algae were isolated from samples of biological soil crusts taken from arid regions of the southwestern USA, as part of the Biotic Crust Project (http://hydrodictyon.eeb.uconn.edu/bcp/). Phylogenetic relationships among the desert algae were determined using previously published 18S rDNA or ITS rDNA sequence data (Lewis & Flechtner 2004; Lewis & Lewis 2005). This information was used to pair the desert algae with their closest aquatic relatives available in culture collections or in culture in our lab (Table 1).

**DNA extraction, PCR, sequencing**

18S rDNA sequence data from three of the aquatic green algae were collected specifically for this study. Isolates of *Scenedesmus platydiscus* (UTEX 2457), *Chlamydomonium* sp. (MLO301CT) and *Chlorella* sp. (UTEX 318) were grown on Bold’s Basal Medium (Bold 1949) agar slants under a 12:12 L/D cycle at 20 °C. Cells were scraped off the slants and concentrated by centrifugation at 3000 g. Genomic DNA was extracted from the algal cells using a modified CTAB extraction that included grinding the algal cells in sterile sand (Shoup & Lewis 2003). The 18S rDNA region was amplified using the primers SSU1 and SSU2, and the resulting fragments were sequenced directly using amplification and internal primers, including 284F and 1081R (Phillips & Fawley 2000), or others listed in Shoup & Lewis (2003). Three new primers (660F 5′ TATGGTGAGTACTGCTATGGC3′, 817R 5′ AGTCCTATCGTGTTATCCATGC3′ and 918F 5′ TGAAAGACGAACTACTGCG3′) were designed in order to allow sequencing through introns in the 18S rDNA gene of UTEX 2457. Double-stranded PCR products were sequenced in 10 µL volumes with the PRISM system (Applied Biosystems, Inc., Foster City, CA, USA) using the manufacturer’s directions. Chromatograms from individual sequencing runs were first trimmed, then assembled into consensus sequences in Sequencher (GeneCodes Corp., Inc., Ann Arbor, MI).

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*The GenBank accession numbers listed in boldface indicate 18S rDNA data that are new to this study.*

*Isolates from the Biotic Crust Project, except for those labelled with the University of Texas Culture Collection of Algae (UTEX) or the Sammlung von Algenkulturen der Universität Göttingen (SAG) acronym.

*This isolate was associated with the original UTEX 318, but is not Microthamnion.*

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Phylogenetic analyses

The 18S rDNA sequences from the 11 green algae isolates listed in Table 1 were aligned by eye in the text editor of PAUP* (Swofford 2002), excluding the intron sequences. In addition to the ingroup sequences, in order to determine the placement of the root for the comparative analysis, the 18S rDNA sequences of two prasinophycean algae were used as outgroup taxa (Nephroselmis olivacea, GenBank accession number X74754, and Tetraselmis striata, X70802). The final 18S alignment consisted of 1770 characters, 97 of which were excluded from the analysis because they could not be aligned with certainty. Of the remaining 1673 characters, 133 were parsimony informative, and 1365 constant. The 18S alignment consisted of 1770 characters, 97 of which were excluded from the analysis because they could not be aligned with certainty. Of the remaining 1673 characters, 133 were parsimony informative, and 1365 constant. The alignment will be made available from TreeBASE (http://www.treebase.org/).

Prior to the maximum likelihood (ML) analyses, the data set was analysed using MODELTEST 3.06 (Posada & Crandall 1998). The favoured substitution model for this alignment of 18S data was determined to be TrN + I + G. Parameter values were set as follows: relative base frequencies (πA = 0.2559, πC = 0.2162, πG = 0.2698, πT = 0.2581); relative rate matrix (rAC = 1.0000, rAG = 2.4720, rAT = 1.0000, rCG = 1.0000, rCT = 5.1053, rGT = 1.0000); gamma shape, 0.6267, and proportion of invariant sites = 0.5048. ML analyses were performed using PAUP* 4.b.10 (Swofford 2002) for UNIX. ML analyses used heuristic searches with 10 random additions of taxa, each followed by TBR branch swapping. Bootstrap analysis (Felsenstein 1985) included 1000 replicates, with a single random addition of taxa for each replicate, and under the same model as was used for the heuristic searches.

Bayesian analyses utilized MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). Two independent runs were performed under the GTR + I + G model (determined to be the best fitting model using MrModeltest v2, Nylander (2004). Each run began with an independent random starting tree and extended two million generations. Each run employed three heated chains (temperature parameter of 0.2) in addition to the cold chain. A flat Dirichlet prior for relative nucleotide frequencies and relative rate parameters, a discrete uniform prior for topologies, and an exponential distribution (mean of 1) for the gamma shape parameter and all branch lengths were used. Trees were sampled every 100 generations, yielding 2000 sampled trees per run. History plots of the overall likelihood scores were plotted to determine which trees were to be excluded as burnin. Results were highly consistent between the two runs, suggesting that each run was long enough to achieve stationarity. Convergence was assessed by comparing splits included in majority-rule consensus trees of each run separately. The trees after burnin from both runs were combined to generate a 50% majority-rule consensus tree. The estimated posterior probability of a given split is shown on the ML tree (Fig. 1).

Figure 1. Maximum likelihood (ML) phylogenetic tree of chlorophycean and trebouxiophycean algae used in the desiccation experiments, estimated from 18S rDNA sequences obtained from algae listed in Table 1. Desert and aquatic algae are indicated by the letters D and A, respectively. Prasinophyte taxa Nephroselmis and Tetraselmis were used to orient the tree. Upper-case text indicates the isolate numbers identifying algal isolates. Text in parentheses indicates the Genbank accession number for the sequences associated with the algal isolate. Boxes associated with nodes of the tree indicate ML bootstrap support (upper number) and Bayesian posterior probabilities (lower number). Details of the tree estimation are described in Materials and Methods.
Culture conditions

Algae were grown in Erlenmeyer flasks as aqueous unicellular cultures in a solution of Bold’s Basal medium supplemented with micronutrients (Trelease & Trelease 1935; Bold 1949) and were allowed to colonize the surface of porous glass beads (Siran carriers 1–2 mm diameter; Jaeger Biotech Engineering, Inc., Costa Mesa, CA, USA) covering the bottom of the flask. Cultures were maintained in an Enconair model GR-27 walk in growth room (Enconair, Winnipeg, Manitoba, Canada) under a 12:12 L/D cycle at a constant temperature of 20 °C, a daytime photosynthetic photon flux density (PPFD) of 130 μmol photons m⁻² s⁻¹ and were bubbled continuously with ambient air. Once colonized, the algae-covered beads were transferred to small plexiglass containers sized to fit snugly into a holder underneath the fiber optic from a PAM-2000 chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) used for all fluorescence measurements. These containers were made from square pieces of 0.635 cm thick plexiglass measuring 1.905 cm on a side. A 1.27 cm diameter hole was bored through the centre, and the bottom was covered in fine mesh to create the floor of the container. Nine containers fit easily into a Petri dish chamber.

Experimental design

The experiment utilized a two-way factorial design in which algal samples were exposed to varying durations of desiccation and varying degrees of illumination during desiccation. Recovery of photosynthetic ability was assessed in rehydrated algae by measuring the photosynthetic quantum yield (efficiency of light utilization by photosystem II [PSII]) using chlorophyll fluorescence (Genty, Briantais & Baker 1989). Recovery of photosynthetic activity was initially measured in algae that were rehydrated in darkness, and later measured in the same algal samples after they were re-illuminated. This allowed us to compare differences in the sensitivity of photosynthesis to desiccation and illumination in the desert and aquatic algae under study.

Each of the 11 algal taxa was assigned to three desiccation treatments (24 h, 7 d and 4 weeks dry) and three illumination treatments (dark, intermediate intensity and full intensity). Each treatment combination was replicated three times for a total of 27 samples for each of the 11 algae, resulting in 297 plexiglass sample containers housing algae on glass beads. Within each illumination treatment, replicate samples assigned to desiccation treatments were distributed across three separate Petri dish chambers in which drying was accomplished by pumping ambient air at 25% relative humidity (RH) via tubing connected to aquarium pumps.

In the ‘dark’ treatment, drying chambers were covered in aluminum foil to exclude light during desiccation and storage. In the two ‘illuminated’ treatments (intermediate intensity and full intensity), drying chambers were left exposed to the illumination regime under which the algae were grown in aqueous culture (130 μmol photons m⁻² s⁻¹ PPFD). Exposure to intermediate light intensity was achieved by allowing the uppermost layer of beads to self-shade lower bead layers. Removing the upper layer immediately prior to rehydration allowed access to algae that had been exposed to light levels intermediate between those of the dark treatment and the upper bead layer of the full intensity treatment. This removal of beads increased the sample fiber optic distance by 2–3 mm and reduced the intensity of the saturating flash for these samples. Examining the fluorescence induction kinetics obtained from these samples, however, showed that fluorescence reached a peak or plateau, indicating that the samples were receiving flashes of sufficient intensity to fully saturate the algal photosystems. Algae were dried and stored under their respective illumination conditions for the duration of their respective desiccation intervals.

Spot checks of the algae following 12–24 h of exposure to the drying air stream showed that photosynthetic quantum yield of the upper bead layers in both the dark and full illumination treatments had declined to <0.1, indicating a loss of photosynthetic activity. Algae were then allowed to remain dry for an additional 24 h, 7 d or 4 weeks in the growth chamber.

Following desiccation intervals of 24 h, 7 d or 4 weeks, the algae were rehydrated by adding water to the Petri dish housing the algae-covered beads in their plexiglass sample containers. Water was allowed to wick up through the mesh container bottom and throughout the algae-covered beads by capillary action. This procedure minimized the physical disturbance of the algae-covered beads during rehydration. Rehydration took place initially in darkness, and the recovery of photosynthetic quantum yield was measured for each plexiglass container 1, 24 and 48 h following the onset of rehydration in darkness. Each chamber was secured at a constant distance underneath the fiber optic of a PAM-2000 fluorimeter (Heinz Walz GmbH), and measurements were gathered as described further. Following rehydration for 48 h in the dark, the algae were re-illuminated at 130 μmol photons m⁻² s⁻¹ PPFD on a regular 12:12 day–night cycle, and photosynthetic quantum yields were measured following illumination of rehydrated algae for 1 and 5 d. Prior to measuring quantum yields of re-illuminated samples, the algae were dark adapted for 6–8 h.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements (Fᵥ, Fm, and Fv/Fm) following standard nomenclature from van Kooten & Snel (1990) and Maxwell & Johnson (2000) were made using the measurement system described in detail in Gray, Cardon & Lewis (2006). This system uses a PAM-2000 portable fluorometer (Heinz Walz GmbH) and a CR10X data logger (Campbell Scientific, Inc., Logan, UT, USA) to implement a protocol for simultaneously recording rapid fluorescence induction kinetics, changes in steady-state fluorescence and an improved method for measuring the true maximum fluorescence (Fm) in algae. The measuring light intensity was set to the lowest setting available with the PAM-2000, corresponding to ~0.06 μmol photons m⁻² s⁻¹ and...
modulated at a frequency of 600 Hz. Saturation pulses were delivered at the lowest intensity halogen setting (≈1450 µmol photons m⁻² s⁻¹) and lasted for either 0.8 or 1.0 s. All fluorescence measurements were made on the algae following a dark adaptation period of at least 6–8 h.

During fluorescence measurements, plexiglass containers containing algae-covered beads were transferred in darkness to a sealed plexiglass measuring cuvette and were placed into a fixed bracket. Air was directed over the surface of the beads while they were held in place. A hole in the lid of the cuvette admitted the PAM-2000 fiber optic to a glass cover slip window directly above the algae. This arrangement allowed repeatable placement of the fiber optic at a fixed distance from the algae between sampling periods. Maintaining such a fixed and repeatable geometry is important for interpreting changes in $F_o$ and $F_m$ because the fluorescence signal obtained from a sample is proportional to the sample to fiber optic distance. In preliminary experiments, by repeatedly removing and replacing the same algal sample between fluorescence measurements, we were able to show that repeated $F_o$ measurements taken on a single sample varied by less than 2% using this cuvette system (data not shown). This allowed us to track changes in absolute levels of fluorescence ($F_o$ and $F_m$), as well as the ratio measurement $F_o/F_m$ (which is not affected by changes in fiber optic to sample distance) during the experiment.

Desiccation dynamics

In order to determine the rate and degree of drying experienced by the algal samples during desiccation, a drying curve was measured for several replicate algal samples identical to those used in the desiccation experiment. After placing a plexiglass container of fully hydrated algae-covered beads in the fluorescence measuring cuvette described earlier, dry air at 25% RH was passed into the cuvette and across the surface of the beads. Exhaust gas from the cuvette was routed to a LI-6262 infrared gas analyser (IRGA) (LI-6262, Li-Cor Inc., Lincoln, NE, USA), which measured water vapour content of the air leaving the cuvette. Both quantum yield and IRGA readings were monitored at 30 min intervals for 24 h. This allowed us to observe the dynamics of water loss from the system and changes in the quantum yield of the algae during the drying process.

Statistical analyses

Analyses were carried out in two major ways, one incorporating phylogenetic information and one that did not.

Overall patterns of fluorescence and photosynthetic recovery (ignoring lineages) were analysed with analysis of variance (ANOVA) using the GLM procedure of the statistical package SAS (1990). The original algal habitat (desert/aquatic), illumination level during desiccation (darkness, intermediate intensity and full intensity), length of desiccation (24 h, 7 d, 4 weeks), and time since rehydration (1 h dark, 24 h dark, 48 h dark, 1 d re-illuminated, 5 d re-illuminated) were treated as fixed main effects. Because the same algal samples were sampled repeatedly during rehydration, the time since rehydration effect was also treated as a repeated factor in the analysis. The algal ‘species’ was treated as a random blocking factor nested within the habitat main effect.

Comparative analyses were used to investigate the associations between habitat of origin and desiccation tolerance, and habitat of origin and resistance to photodamage while desiccated, using phylogenetically corrected generalized least squares (PGLS) regression as implemented in version 4.6b of the software package COMPARE (Martins 2004). PGLS allows testing of correlations between the characters in a group of species (e.g. traits and habitat) taking into account the underlying phylogenetic relationships among taxa. In PGLS, the parameter alpha sets the rate of adaptation of a trait to the optimum. When alpha is large, traits move instantly to new optima set by natural selection, and there is no correlation between ancestors and descendants. Under conditions of large alpha, the analysis approximates a non-phylogenetically corrected least squares regression (TIPS). TIPS assumes (potentially incorrectly) that all species in the analysis are evolutionarily independent units of observation. When alpha is small, PGLS approximates Felsenstein’s independent contrasts (FIC), and phylogeny is a strong determinant of correlations among characters. The PGLS ML estimate of alpha is usually intermediate to these two extremes (‘PGLS’).

To examine the differential impact of desiccation and illumination during desiccation on photosynthetic performance in aquatic and desert algae, we derived two indices of damage. The desiccation damage index (DDI) measures the fraction of initial aqueous quantum yield lost following desiccation in darkness. This is described in Eqn 1 where \( Yield_{\text{dark(avg)}} \) represents the average quantum yield of an algal taxon obtained from a random subsample \((n = 3)\) of dark-adapted replicates prior to application of the desiccation treatment; \( Yield_{\text{dark(dry)}}(i) \) is the quantum yield of an individual rehydrated algal sample that was previously dried and stored in darkness, where the subscript \((i)\) indicates different replicate samples. The total number of samples measured is indicated by \( j \).

\[
DDI = \frac{1}{j} \sum_{i=1}^{j} \frac{Yield_{\text{dark(avg)}} - Yield_{\text{dark(dry)}}(i)}{Yield_{\text{dark(avg)}}} \quad (1)
\]

The photodamage index (PDI) measures the additional fraction of initial quantum yield that is lost when desiccated algae are also exposed to illumination while they are dry. This is described by Eqn 2, where \( Yield_{\text{dark(avg)}} \) represents the average quantum yield of all dark-adapted algae samples within a taxon prior to application of the desiccation treatment \((n = 27)\); \( Yield_{\text{dark(dry)}}(i) \) represents the average quantum yield of rehydrated algal isolate previously dried and stored in darkness \((n = 3)\); \( Yield_{\text{illum(dry)}}(i) \) is the quantum yield of an individual rehydrated algal sample that was previously dried and stored under illumination, where the subscript \((i)\) indicates different replicate samples. The total number of samples measured is indicated by \( j \).

\[
\text{PDI} = \frac{1}{j} \sum_{i=1}^{j} \frac{Yield_{\text{dark(dry)}}(i) - Yield_{\text{illum(dry)}}(i)}{Yield_{\text{dark(avg)}}} \quad (2)
\]
Both DDI and PDI scale algal responses to their initial quantum yield, thereby allowing a fair comparison among algae that may have inherently different quantum efficiencies of light utilization even when growing in aqueous culture.

RESULTS

Sequencing and phylogenetic reconstruction

The resulting 18S rDNA regions ranged from 1664 to 5293 nucleotides in length. The *S. platydiscus* sequence contains eight putative group IA introns, in positions 125, 989, 1534, 1976, 2840, 3484, 4278 and 4909 of the 18S rDNA region. The entire fragment was PCR amplified in segments rather than as a single amplification product. A 43-nucleotide region of the 18S rDNA exon, present in the other algae sequences, was not determined because of sequencing difficulty. The 43 undetermined positions, corresponding to positions 4094–4135 in the sequence, were designated as the nucleotide ambiguity code ‘N’ in the alignment. As this region was not determined by sequencing, it is possible that additional inserted sequences are present in the rDNA gene of *S. platydiscus*. The newly obtained 18S consensus sequences were subjected to BLAST searches (Altschul et al. 1990) in order to screen for contaminant sequences prior to phylogenetic analyses. The 18S sequences from these isolates were deposited in GenBank under accession numbers EF159950–EF159952.

ML analysis produced a phylogenetic tree with a score of −lnL = 5066.9385 that groups the ingroup taxa into three major lineages, each containing desert and aquatic derived taxa (Fig. 1). Two of these lineages correspond to the clockwise and directly opposed flagellar apparatus orientation groups in Chlorophyceae, and the third lineage contains members of Trebouxiophyceae (Friedl 1995).

Desiccation dynamics

Water content of the air leaving the fluorescence cuvette reached equilibrium shortly following placement of the algal sample in the fluorescence cuvette. Humidity in the cuvette remained constant for several hours, after which water content of the air in the cuvette began to decline precipitously over a 3 h period until the water content of the exhaust air equilibrated at a dewpoint of 2 °C (relative humidity of 25%). This pattern suggests an initial period in which evaporation occurred from bulk water surrounding the beads and was sufficient to maintain constant high humidity in the chamber (Fig. 2). During this time, quantum yield from the algae was very high. This was then followed by a period of declining cuvette humidity as the remaining water evaporated from the beads and from the algal cells. As the chamber humidity declined, quantum yield of the algal sample also declined; however, the decline in quantum yield was delayed and did not take place until chamber humidity had declined significantly. This delay might indicate a threshold water content below which algal physiology was adversely impacted. Once chamber humidity had equilibrated with that of the incoming air (25% RH), algal quantum yields also equilibrated at yields below 0.1, indicating a loss of photosynthetic activity in the desiccated algae. The pattern of changes in quantum yield observed in this drying curve closely matched the pattern of quantum yield measurements observed in algae desiccated in Petri dishes during the desiccation experiment. In both cases, quantum yields began to decline after 12 h of exposure to a desiccating airstream, and photosynthetic activity was lost within 24 h.

Visible impacts of desiccation and illumination: photobleaching

Following desiccation in darkness, dry beads colonized by aquatic and desert algae remained extensively pigmented regardless of the length of time they were desiccated (24 h, 7 d and 4 weeks). Beads colonized with desert and aquatic algae also remained heavily pigmented when dried under illumination for either 24 h or 7 d. However, following 4 weeks of desiccation under illumination, all the aquatic algae were noticeably bleached of color compared to their counterparts dried in darkness. This photobleaching was also present to varying but lesser degrees in the desert algae, with desert SEV3-VF49 and SEV2-VF1 showing the least loss of color compared with samples dried under darkness. Photobleaching was limited to the uppermost layers of beads, and darkly pigmented surfaces were present on beads beneath the bleached top layer and frequently on the underside of a bleached bead.
Recovery of photosynthetic quantum yield during rehydration of algae dried in darkness

Photosynthetic quantum yields measured on dry algae were all <0.1, regardless of pigmentation, indicating a loss of photosynthetic activity. The ability of algae to recover photosynthetic quantum yield when rehydrated in darkness declined as algae remained dry for progressively longer times, as indicated by the significant effect of dry time (Table 2). In aquatic algae, most of the ability to recover quantum yield upon rehydration was lost after 24 h of desiccation, while desert algae still recovered some quantum yield even following 4 weeks of desiccation in darkness (Fig. 3). These differences in the behaviour of desert and aquatic algae suggest that adaptation to desiccation may be species-specific and may be related to evolutionary history.

Table 2. Results of analysis of variance (ANOVA) from the desiccation experiment examining differences in photosynthetic quantum yield as a function of habitat of origin (habitat), illumination treatment (light), length of desiccation (dry) and rehydration interval (RH time)

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MS</th>
<th>Error term</th>
<th>MSE</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
<td>1</td>
<td>15.8199</td>
<td>Alga (habitat)</td>
<td>0.4354</td>
<td>36.33</td>
<td>0.0002</td>
</tr>
<tr>
<td>Light</td>
<td>2</td>
<td>1.13361</td>
<td>Alga × light(habitat)</td>
<td>0.059104</td>
<td>19.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dry</td>
<td>2</td>
<td>1.12877</td>
<td>Dry × alga(habitat)</td>
<td>0.049507</td>
<td>22.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RH time</td>
<td>4</td>
<td>3.44088</td>
<td>RH × alga(habitat)</td>
<td>0.121457</td>
<td>28.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Habitat × light</td>
<td>2</td>
<td>0.18319</td>
<td>Alga × light(habitat)</td>
<td>0.059094</td>
<td>3.10</td>
<td>0.0697</td>
</tr>
<tr>
<td>Habitat × dry time</td>
<td>2</td>
<td>0.39981</td>
<td>Dry × alga(habitat)</td>
<td>0.049482</td>
<td>8.08</td>
<td>0.0031</td>
</tr>
<tr>
<td>Habitat × RH time</td>
<td>4</td>
<td>0.13173</td>
<td>RH × alga(habitat)</td>
<td>0.121975</td>
<td>1.08</td>
<td>0.3787</td>
</tr>
<tr>
<td>Dry × RH</td>
<td>5</td>
<td>0.02178</td>
<td>Dry × RH × alga(habitat)</td>
<td>0.008543</td>
<td>2.55</td>
<td>0.0409</td>
</tr>
<tr>
<td>RH × light</td>
<td>8</td>
<td>0.08551</td>
<td>RH × alga(habitat)</td>
<td>0.07027</td>
<td>12.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Habitat × dry × light</td>
<td>4</td>
<td>0.04958</td>
<td>Dry × alga × light(habitat)</td>
<td>0.018297</td>
<td>2.71</td>
<td>0.0454</td>
</tr>
<tr>
<td>Habitat × RH × light</td>
<td>8</td>
<td>0.08965</td>
<td>RH × alga × light(habitat)</td>
<td>0.007027</td>
<td>12.76</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dry × RH × light</td>
<td>10</td>
<td>0.00733</td>
<td>Dry × RH × alga × light(habitat)</td>
<td>0.003374</td>
<td>3.41</td>
<td>0.0008</td>
</tr>
<tr>
<td>Habitat × dry × RH × light</td>
<td>10</td>
<td>0.01150</td>
<td>Dry × RH × alga × light(habitat)</td>
<td>0.003374</td>
<td>3.41</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

Table 3. Results of phylogenetic comparative analyses examining the correlation between habitat of algal origin and recovery of photosynthetic quantum yield

<table>
<thead>
<tr>
<th>Rehydration period</th>
<th>Desiccation period</th>
<th>TIPS</th>
<th>PGLS (alpha)</th>
<th>FIC</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark 24 h</td>
<td>24 h</td>
<td>-0.95</td>
<td>-0.95 (15.50)</td>
<td>-0.97</td>
<td>-0.58 ± 0.13*</td>
</tr>
<tr>
<td></td>
<td>7 d</td>
<td>-0.90</td>
<td>-0.90 (15.50)</td>
<td>-0.94</td>
<td>-0.55 ± 0.16*</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>-0.92</td>
<td>-0.93 (6.06)</td>
<td>-0.96</td>
<td>-0.50 ± 0.13*</td>
</tr>
<tr>
<td>Illuminated 24 h</td>
<td>24 h</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 d</td>
<td>-0.65</td>
<td>-0.66 (5.44)</td>
<td>-0.69</td>
<td>-0.35 ± 0.26*</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>-0.65</td>
<td>-0.72 (0.957)</td>
<td>-0.76</td>
<td>-0.35 ± 0.22*</td>
</tr>
<tr>
<td>PDI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark 24 h</td>
<td>24 h</td>
<td>0.59</td>
<td>0.59 (15.50)</td>
<td>0.45</td>
<td>0.09 ± 0.08*</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>0.61</td>
<td>0.66 (1.88)</td>
<td>0.74</td>
<td>0.21 ± 0.15*</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>0.93</td>
<td>0.93 (15.50)</td>
<td>0.96</td>
<td>0.45 ± 0.11*</td>
</tr>
<tr>
<td>Illuminated 24 h</td>
<td>24 h</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 d</td>
<td>-0.29</td>
<td>-0.37 (2.95)</td>
<td>-0.55</td>
<td>-0.09 ± 0.15 NS</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>-0.07</td>
<td>0.06 (15.50)</td>
<td>-0.14</td>
<td>0.01 ± 0.14 NS</td>
</tr>
</tbody>
</table>

Results are presented for rehydration in darkness for 24 h and after re-illumination for 1 d in algae dried for 24 h, 7 d, and 4 weeks. Notes: The desiccation damage index (DDI) indicates the fraction of the initial aqueous quantum yield that was lost as a result of desiccation. The photodamage index (PDI) indicates the additional fraction of aqueous quantum yield that was lost as a result of illumination. *Comparisons yielding significant correlations. PGLS, phylogenetically generalized least squares using the maximum likelihood (ML) estimate of alpha. FIC, approximation to Felsenstein independent contrasts; TIPS, non-phylogenetically corrected generalized least squares regression; NS, non-significant results; NA, no data available.

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aquatic algae were statistically significant as indicated by a significant habitat effect (Table 2).

Within 1 h of the onset of rehydration in the dark, aquatic algae desiccated for 24 h in darkness had recovered very little (Fig. 3a). Following 24 h of rehydration, quantum yields of aquatic algae were only slightly greater than yields 1 h post rehydration (Fig. 3a,b). In contrast, desert algae desiccated for 24 h in darkness exhibited rapid recovery of photosynthetic quantum yield upon rehydration, reaching 56–91% of their initial aqueous culture yield within 1 h (Fig. 3c). After 24 h of rehydration in darkness, little additional recovery of yield was observed (Fig. 3d).

Recovery of photosynthetic quantum yield in algae illuminated during desiccation

Recovery of photosynthetic quantum yield following rehydration was consistently lower in algae dried under illumination than in algae dried in darkness for equal lengths of time, as indicated by a significant treatment effect in Table 2. To ease visual comparisons in Fig. 4, results from all aquatic and desert species, respectively, have been grouped. Grouped data from dark-dried algae are shown in black bars in Fig. 4, alongside data from beads that were fully exposed to light during desiccation (full illumination = white bars) and algae on beads that were below the top-most bead layer during desiccation in the light (intermediate illumination = grey bars). Data are separated into panels based on the length of time the algae remained desiccated. Rehydration treatments (e.g. 1 and 24 h rehydration in the dark, as in Fig. 3, along with further hydration in the light) are indicated along the x-axes of Fig. 4.

Aquatic algae had lost nearly all ability to recover quantum yield following desiccation for as little as 24 h (Fig. 3a,b), and illumination during desiccation tended to reduce their ability to recover quantum yield still further (Fig. 4a–c). The negative effect of light exposure during desiccation was particularly pronounced in the desert algae (Fig. 4d–f, grey and white bars), all of which had shown rapid recovery to high quantum yields upon rehydration for even just 1 h following all lengths of desiccation in darkness (Figs 3c,d & 4d–f, black bars). Over time, as desert and aquatic algae were rehydrated first in the dark and then with supplemental light, the effect of having been desiccated in light became less obvious (Fig. 4), although the aquatic algae were much more variable in their recovery in the light (Fig. 4b,c). Unfortunately, for the algae dry only 1 d, recovery of quantum yield was not tracked after 48 h in dark rehydration (Fig. 4a,d). Following 4 weeks of desiccation under illumination, the upper layer of most of the desert algae exhibited no recovery even after 24 h of rehydration in darkness (Fig. 4f), although these same algal species recovered significant quantum yields when dried 4 weeks in darkness (Figs 3c,d & 4f, black bars). Examining quantum yields of algae on beads located beneath the uppermost bleached surface layer revealed levels of recovery that were intermediate between those of algae dried in darkness and the upper layers of algae dried under illumination (Fig. 4, grey bars).

Recovery of photosynthetic quantum yield in algae re-illuminated following rehydration

The highly variable recovery among diverse aquatic algae exposed to light during rehydration (right-most groups of
Photorecovery after desiccation of green microalgae

Aquatic algae

Desert algae

Figure 4. Recovery of photosynthetic quantum yield during rehydration of aquatic (a–c) and desert (d–f) algae exposed to various light intensities during desiccation. Recovery following desiccation for 24 h is shown in (a) and (d); following 7 d is shown in (b) and (e), or following 4 weeks is shown in (c) and (f). Bars represent quantum yields of rehydrated algae averaged over all aquatic (a–c) or desert (d–f) isolates. Error bars represent ±1 SD with n = 15 (aquatic) or n = 18 (desert). Data were not collected for algae re-illuminated following desiccation for 24 h (a,d). All measurements were made following a 6–8 h period of dark adaptation. Key to symbols: black bars = algae dried in darkness, grey bars = upper bead layer of algae dried under illumination, white bars = lower bead layer of algae dried under illumination.

Bars, Fig. 4b–c) is further explored in Fig. 5a,b. The aquatic algae UTEX 11 and UTEX 318, which showed very low recovery of photosynthetic quantum yield when rehydrated in darkness (Figs 3a,b & 5a,b), exhibited dramatic increases in quantum yield when they were re-illuminated for just 1 d during rehydration (Fig. 5a,b). UTEX 318 showed almost complete recovery of photosynthetic quantum yield following 5 d of illumination during rehydration (Fig. 5a,b, hatched bars). In contrast, MLO301CT showed no recovery from desiccation, no matter the rehydration treatment, and SAG 2006 and UTEX 2457 were intermediate (Fig. 5a,b). Upon re-illumination (after rehydration), the dramatic increases in quantum yield observed for UTEX 318, UTEX 11 and the intermediate increase observed for UTEX 2457 coincided with declines in \( F_o \) of ~50% or more relative to pre-illumination levels, whether algae had been desiccated previously in the dark or under light (Fig. 6a,b). These declines in \( F_o \) were accompanied by losses of pigmentation easily visible to the naked eye 24 h after re-illuminating the rehydrated algae (\( F_o \) was measured after overnight dark adaptation of algae). A contrasting pattern of gradual \( F_o \) decrease during rehydration for MLO301CT and SAG 2006 was observed, particularly after desiccation in the dark (Fig. 6a). These two algae did not exhibit easily visible losses of pigmentation upon rehydration, although SAG 2006 did exhibit a drop in \( F_o \) of nearly 50% upon re-illumination when it had been desiccated in the light (Fig. 6b).

Patterns of recovery for desert algae dried under illumination were quite different from those for aquatic algae (Fig. 5c,d). All desert algae dried under illumination for 4 weeks, and then rehydrated first in the dark, then with illumination, recovered a large proportion of the original quantum yield (Fig. 5d). In all but one case (SEV3-VF49), the majority of recovery occurred after light was applied during rehydration (Fig. 5d, hatched bars). This behaviour parallels that of aquatic alga UTEX 318 (Fig. 5b). In contrast, desert algae dried in the dark (Fig. 5c) recovered a much higher proportion of the original quantum yield even after just 1 h rehydration in the dark. Application of light during rehydration was associated with further recovery (Fig. 5c, hatched bars); however, the relatively large recovery of photosynthetic quantum yield attained by the desert algae upon rehydration in darkness (Figs 3c,d; 4d–f & 5c) made these increases less pronounced than those observed in the aquatic algae (Figs 4b,c & 5a,b). Unlike in the aquatic algae (Fig. 6a), \( F_o \) in dark-desiccated desert algae overall remained much more constant during rehydration, even when illumination was applied (Fig. 6c).

As noted previously, desert algae dried under illumination exhibited only partial recovery of quantum yield when
rehydrated in darkness (Fig. 4d–f). Following 4 weeks of desiccation under illumination, three of the desert algae (BC2-1, CNP2-VF25 and BC4-VF9) exhibited no measurable recovery of quantum yield when rehydrated in darkness for up to 48 h (Fig. 5d). Yet, upon re-illumination, photosynthetic quantum yield in BC2-1, CNP2-VF25 and BC4-VF9 increased significantly (Fig. 5d, hatched bars), following a pattern similar to that exhibited by the aquatic alga UTEX 318 (Fig. 5b). The swift recovery of quantum yield when light was applied to BC2-1, CNP2-VF25 and

Figure 5. Recovery of photosynthetic quantum yield following rehydration of aquatic (a,b) or desert (c,d) algae previously desiccated for 4 weeks in darkness (a,c) or under full intensity illumination of 130 μmol m⁻² s⁻¹ (b,d). Columns represent average quantum yield following 6–8 h dark adaptation. Error bars represent ±1 SD with n = 3. Key to symbols: black bars = pre-desiccation, dark grey = algae rehydrated for 1 h in darkness, medium grey = algae rehydrated for 24 h in darkness, light grey = algae rehydrated for 48 h in darkness, right hatched = algae re-illuminated for 1 d, and left hatched = algae re-illuminated for 5 d.

Figure 6. Recovery of dark-adapted steady-state fluorescence (F₀) following rehydration of aquatic (a,b) or desert (c,d) algae previously desiccated for 4 weeks in darkness (a,c) or under full intensity illumination of 130 μmol m⁻² s⁻¹ (b,d). Columns represent average F₀ following 6–8 h dark adaptation. Error bars represent ±1 SD with n = 3. Key to symbols: black bars = pre-desiccation, dark grey = algae rehydrated for 1 h in darkness, medium grey = algae rehydrated for 24 h in darkness, light grey = algae rehydrated for 48 h in darkness, right hatched = algae re-illuminated for 1 d, and left hatched = algae re-illuminated for 5 d.

Quantum yield (F_v/F_m)
BC4-VF9 during rehydration was accompanied by declines in $F_o$ (Fig. 6d), and these declines in $F_o$ occurred in conjunction with visibly noticeable losses of pigmentation that took place upon re-illumination. However, both the declines in $F_o$ and the loss of color in the desert algae were less severe upon re-illumination than those observed in the aquatic algae. Interestingly, these three desert algae (BC2-1, CNP2-VF25 and BC4-VF9) were also the three desert taxa most visibly bleached by desiccation in the light. When dried under illumination, the three other desert algae (SEV2-VF1, SEV3VF49 and BC8-8) exhibited a delayed and/or low level of quantum yield recovery during rehydration in darkness (Fig. 5d); re-illumination enhanced their recovery, but $F_o$ did not exhibit abrupt declines following re-illumination (Fig. 6d).

**Ecological differences**

Comparing the impact of desiccation on the recovery of quantum yield in desert algae with that of aquatic algae shows that there is a very strong phylogenetically corrected correlation ($r = 0.9–0.95$) between habitat of origin and the capacity to recover quantum yield when rehydrated in darkness (Fig. 7 & Table 3). These correlations were significant for all periods of desiccation. However, the strength of this correlation declined markedly ($r = 0.65$) upon re-illumination (Table 3), indicating that illumination can ameliorate some of the damage caused by desiccation. These patterns are clearly evident in Fig. 7a, which shows that DDI is consistently higher in aquatic algae than in desert algae, but that DDI declines in the aquatic algae following re-illumination.

In addition to the damage incurred by the desert algae upon desiccation, desert algae also lost an additional 25–55% of their initial aqueous quantum yield as a result of being illuminated while dry. These reductions in quantum yield due to illuminating dry algae (Fig. 7b) were comparable in magnitude to the reductions in yield due solely to desiccation (Fig. 7a). In response to re-illumination, PDI of the desert algae declined, exhibiting a response that was similar to that observed for DDI in the aquatic algae, and suggesting that re-illumination of rehydrated algae could facilitate repair of damage caused by illuminating dry desert algae.

In contrast, aquatic algae lost most of their initial aqueous quantum yield following desiccation in darkness, as indicated by very high DDI values (Fig. 7a). Because little residual capacity for photosynthetic recovery was present in aquatic algae following desiccation, illuminating dry aquatic algae could only cause small additional losses in quantum yield, and resulted in very low calculated PDI values (Fig. 7b).

**DISCUSSION**

Unicellular green microalgae are common in microbiotic crusts worldwide. Because they are very small (10–50 μm) and rarely dominant, other photosynthetic organisms from crusts, such as mosses, lichens and the cyanobacteria *Microcoleus* and *Nostoc*, have more often been the focus of physiological studies. Further, because desert green algae are morphologically very similar to one another, their impressive diversity has only been revealed recently using molecular methods (e.g. Lewis & Lewis 2005), and exploration of green algal contributions to crusts has focused on them as a group, not at the species level. This impressive diversity has been shown by Lewis & Lewis (2005) to be associated with at least 14 evolutionarily independent transitions from aquatic to desert habitats. This fact, coupled with the algae’s simple coccoid morphology and their persistence in what would appear to be very harsh environments, suggests these algae offer an excellent opportunity to explore physiological tolerance and survival strategies that have evolved in nature among unicellular eukaryotes making the transition from water to land. By taking into account underlying
phylogenetic relationships among algae, modern phylogenetic comparative methods aid in teasing out whether a congruent pattern of occurrence in two characters (e.g. a physiological trait and habitat) actually represents correlated evolutionary change in the two characters, or a chance pattern of association (Martins 2004). Stronger evidence for correlated evolution between characters is provided by multiple independent coevolutionary events. Less convincing is a single evolutionary origin of an association between characters, the presence of which in many species may be easily explained by historical relationship. In our case, having multiple data points, each representing an independent origin of desert organisms, ultimately will support a more conclusive demonstration of correlated evolution of physiological traits and a shift to the desert habitat.

The initial step must be to show that isolates of green algae from deserts are not just transient visitors from aquatic systems – they did not just blow in on the wind or persist following a flood or rainstorm. Molecular evidence suggests they are not transient aquatic visitors (Lewis & Lewis 2005); now the physiological data presented here further strengthen that conclusion. Desert green algae were able to recover substantial photosynthetic quantum yield within 1 h of rehydration (Figs 3c,d & 4d-f), particularly when desiccated in darkness (Figs 4d-f & 5c,d). Aquatic algae were much more sensitive to desiccation (Figs 3a,b; 4a–c; 5a,b; Tables 2 & 3). Interestingly, much of the recovery achieved by the desert algae took place within 1 h of rehydration (Figs 3c & 5c), a length of time that is likely too short for the recovery to be explained by massive synthesis of new proteins and construction of new photosystems (Mattoo, Marder & Edelman 1989; Barber & Andersson 1992). It suggests that the desert algae possess well-developed mechanisms that protect their photosynthetic machinery from desiccation-induced damage. The rapid recovery of photosynthetic processes observed in the desert algae parallels that seen in some species of cyanobacteria (Harel et al. 2004) and mosses (Proctor & Smirnoff 2000). In these organisms, recovery of photosynthetic activity occurred within minutes of rehydration and did not require protein synthesis, leading to the conclusion that recovery was due to the reassembly of PSII from existing photosystem components that were present and intact in the desiccated state (Proctor & Smirnoff 2000; Harel et al. 2004). Determining whether this is the case in desert green algae requires further experimentation.

Despite their ability to withstand desiccation, the desert algae examined in this study were susceptible to photodamage when dry. This result is interesting in that these algae were exposed to rather low intensities of light (130 μmol m⁻² s⁻¹), but they were isolated from desert crusts where incident light would have approached full sun (2000 μmol m⁻² s⁻¹) at the surface. When exposed to light while they were desiccated, the desert algae recovered photosynthetic quantum yield more slowly upon rehydration than they did when dried in darkness, and exhibited a dose dependence of their response to illumination both in terms of the intensity of incident light and the duration of illumination (Fig. 4d–f).

Not all desert algal species behaved similarly, however. For BC2-1, CNP2-VF25 and BC4-VF9, exposure to light during desiccation for 4 weeks led to the complete failure to recover measurable photosynthetic quantum yield even after 48 h of rehydration in darkness (Fig. 5d). Re-illuminating these algae during rehydration stimulated a dramatic increase in quantum yield (Fig. 5d) accompanied by abrupt declines in F₀ (Fig. 6d), which may reflect the re-association or repair of photosystem components and the establishment of a functioning system for dissipating absorbed energy through the electron transport chain. Heightened F₀ has frequently been observed following stress in a variety of photosynthetic organisms (Angelopoulos, Dichio & Xiloyannis 1996; Briantais et al. 1996; Bartoskova, Komenda & Naus 1999; Kouril et al. 2001) and is consistent with a mechanism in which disruption of the electron transport chain reduces the potential for energy dissipation via photochemistry, resulting in increased chlorophyll fluorescence. However, it is also of note that upon re-illuminating the rehydrated desert algae, there was a visible (even to the eye) loss of pigmentation in all samples, and the loss of color was most pronounced in the three algae that exhibited the lowest recovery of quantum yield in darkness (BC2-1, CNP2-VF25 and BC4-VF9). An alternative explanation for the declines in F₀ upon re-illumination of rehydrated algae noted earlier (Fig. 6d), then, may be that F₀ declined as a result of final photodestruction of chlorophyll in a subpopulation of already damaged or dead cells. The remaining fluorescence signal, indicating improved quantum yield upon re-illumination (Fig. 5d), would in this scenario be derived from the surviving cells. Among those desert algae that did recover quantum yield in darkness following long-term desiccation under illumination (SEV3-VF49, SEV2-VF1 and BC8-8), the slow or delayed recovery they exhibited (Fig. 5d) in comparison with dark dried samples (Fig. 5c) was also consistent with a mechanism involving the repair or construction of new photosystems.

Although aquatic algae rehydrated in darkness exhibited little or no recovery of photosynthetic quantum yield (Fig. 3a,b), quantum yield in a subset of these aquatic algal species (UTEX 2457, UTEX 11, UTEX 318) recovered dramatically upon re-illumination (Fig. 5a,b). The concomitant decline in F₀ in these taxa (Fig. 6b) and the visible loss of color noted after re-illumination parallel the patterns in desert BC2-1, CNP2-VF25 and BC4-VF9 desiccated long term in the light (Fig. 5d). Following rehydration in darkness, most of the cells in the UTEX 2457, UTEX 11 and UTEX 318 samples apparently had retained large amounts of chlorophyll, but they lacked functional PSII units able to undergo charge separation (either dead or extremely stressed cells). The presence of large amounts of chlorophyll would have resulted in the high F₀ values observed in the dark, but the lack of charge separation within PSII would have resulted in low F₀ values, little variable fluorescence and hence, low quantum yields (Krause & Weis 1991). Upon re-illumination, this chlorophyll in dead or very...
stressed cells likely was damaged, resulting in the overall decline in \( F_v \). However, at least some of the algal cells in those samples of aquatic isolates appear to have survived desiccation, and fluorescence from those survivors may have dominated the high yield measurements 1 and 5 d after re-illumination (Fig. 5b).

**Ecological patterns and significance**

Within their native habitat, desert crust algae must withstand numerous environmental stresses including periods of intense desiccation, temperature extremes and intense irradiance at the soil surface, potentially for months at a time (Rosentretre & Belnap 2001). This capacity for rapidly recovering photosynthetic ability would be advantageous in allowing these desert algae to take advantage of brief periods of water availability afforded by desert thunderstorms. Examining the cell morphology of these algae in aqueous culture, while desiccated, and after rehydration revealed no visible differences in cell morphology, suggesting that these algae withstand desiccation as vegetative cells rather than as special resting stages or zygote spores.

In contrast to the behaviour of the desert algae, we observed no photosynthetic recovery in closely related aquatic algae rehydrated in darkness, and re-illumination appeared to lead to recovery of only a sub-population of cells from some aquatic taxa (Figs 5a, b & 6a, b). This suggests that the high degree of desiccation tolerance observed in the desert algae is an adaptation to the desert environment rather than an ancestral characteristic of the evolutionary lineages to which the desert algae belong. This view is supported by our comparative statistical analysis through which we show a strong significant correlation between habitat of origin and the ability to recover from desiccation (Table 3). PGLS, TIPS and FIC all produced similar correlation coefficients in Table 3 because, with the suite of species we used in the experiment, underlying lineage-specific traits were not driving results. Had all desert algae been derived from a single transition from water to land by one aquatic ancestor that possessed measurable desiccation tolerance, then contrasting results from PGLS and TIPS analyses would have indicated that the physiological traits inherited by the related desert descendants were driving the correlations between trait and habitat detected in the data.

Although the desert algae proved to be quite resistant to desiccation, the degree to which they were impacted by illumination (photodamage) is somewhat unexpected. The desert algae examined in this study originated from crust samples and clearly must be able to survive months of desiccation and illumination in crusts in the field. One possible explanation for this pattern is that the desert green algae may be able to acclimate to the higher light intensities present under field conditions by synthesizing more photoprotective pigments, such as xanthophyll cycle pigments (Bilger & Björkman 1990; Demmig-Adams & Adams 1992; Gilmore 1997; Buhkho et al. 2001b; Masojidek et al. 2004). Alternatively, the light sensitivity shown in this study may indicate that in the field, these algae occupy micro-environments within the crust where they are protected from damaging light levels. The differential susceptibility to illumination expressed by the taxa examined in this study suggests there may be a complex spatial arrangement of green algal species in the crust structured in response to the attenuation of light with depth through the crust profile. Vertical profiles of organisms are well-known characteristics of biological crusts (Hu et al. 2003; Hoppert et al. 2004); however, because many crust organisms lack readily discernible morphological features, most studies have focused on the stratification of taxa grouped at higher taxonomic levels rather than at the level of species. As noted earlier, the problem of species identification is particularly acute for the eukaryotic green algae. Determining the location of individual species within a crust will require the development of molecular probes for in situ tagging of specific species.

Physical disturbances are well known to have deleterious effects on crusts (Belnap & Eldridge 2001) and would represent an important threat to their green algal assemblages, by potentially exposing light-sensitive algae protecting protected lower crust layers to high levels of illumination. This enhanced illumination could lead to eventual loss of photosensitive taxa and reductions in algal biodiversity in the disturbed crust fragments. In addition to potentially rendering these green algal communities susceptible to disturbance, their apparent sensitivity to illumination may have important implications for designing remediation efforts aimed at regenerating disturbed crusts. One technique that has been used involves inoculating disturbed soil surfaces with cultured crust organisms or a slurry made from soil crust fragments (St. Clair, Johansen & Webb 1986; Belnap 1993). These inoculants have consistently enhanced the rates of soil crust recovery following disturbance, and cyanobacteria/green algae, mosses and lichens show enhanced coverage and biomass in inoculated areas (Belnap 1993; Belnap & Eldridge 2001). Lichens and mosses also show enhanced species diversity (Belnap 1993; Belnap & Eldridge 2001) in inoculated areas. Studies, however, have not distinguished between the cyanobacterial and green algal components within crusts. Our results suggest that spraying green algae cultured from desert crusts directly onto soil surfaces as inoculants may prove problematic for re-establishing these green algae given their susceptibility to photodamage during desiccation.

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