SYNOPSIS. Drying to equilibrium with the air is lethal to most species of animals and plants, making drought (i.e., low external water potential) a central problem for terrestrial life and a major cause of agronomic failure and human famine. Surprisingly, a wide taxonomic variety of animals, microbes, and plants do tolerate complete desiccation, defined as water content below 0.1 g H₂O g⁻¹ dry mass. Species in five phyla of animals and four divisions of plants contain desiccation-tolerant adults, juveniles, seeds, or spores. There seem to be few inherent limits on desiccation tolerance, since tolerant organisms can survive extremely intense and prolonged desiccation. There seems to be little phylogenetic limitation of tolerance in plants but may be more in animals. Physical constraints may restrict tolerance of animals without rigid skeletons and to plants shorter than 3 m. Physiological constraints on tolerance in plants may include control by hormones with multiple effects that could link tolerance to slow growth. Tolerance tends to be lower in organisms from wetter habitats, and there may be selection against tolerance when water availability is high. Our current knowledge of limits to tolerance suggests that they pose few obstacles to engineering tolerance in prokaryotes and in isolated cells and tissues, and there has already been much success on this scientific frontier of desiccation tolerance. However, physical and physiological constraints and perhaps other limits may explain the lack of success in extending tolerance to whole, desiccation-sensitive, multicellular animals and plants. Deeper understanding of the limits to desiccation tolerance in living things may be needed to cross this next frontier.

INTRODUCTION

Drying to equilibrium with even moderately dry air is instantly lethal to most species of animals and plants, making water availability one of the most important ecological factors and evolutionary pressures on terrestrial life. However, there are species of animals, plants, and microbes that do tolerate complete desiccation. Among animals, desiccation tolerance is common in three phyla: nematodes (Wharton, 2003), rotifers (Ricci, 1998; Ricci and Carprioli, 2005), and tardigrades (Wright et al., 1992; Wright, 2001). Tolerance in juveniles is known from two more phyla, in the encysted embryos of one crustacean genus (Clegg, 2005) and in the larva of one species of fly (Kikawada et al., 2005). Among plants, desiccation tolerance is common in bryophytes (Proctor and Tuba, 2002; Olliver et al., 2005) and rare in adult pteridophytes and angiosperms (Porembski and Barthlott, 2000) yet common in their spores, seeds, and pollen (Dickie and Prichard, 2002; Tweddle et al., 2003; Farmsworth, 2004; Illing et al., 2005). The extent of desiccation tolerance remains less well known in prokaryotes and fungi, but many bacteria (Potts, 1994; Guerrero et al., 1999; Billi and Potts, 2002), terrestrial microalgae (Ong et al., 1992; Agrawal and Pal, 2003), and lichens (Palmqvist, 2000; Beckett et al., 2003; de la Torre et al., 2003; Kranner et al., 2003) and some yeasts (Sales et al., 2000) tolerate desiccation, as does at least one intertidal macroalga (Abe et al., 2001).

The taxonomic diversity of tolerant species suggests that the potential to evolve tolerance is widespread, and the obvious advantages of tolerance for survival on land suggest that there should be strong selection for tolerance. The main evolutionary puzzle about desiccation tolerance is therefore why it is not more common. A clue to this puzzle may lie in the morphology and ecology of tolerance: desiccation-tolerant organisms are either small or rare or both. No animals longer than 5 cm tolerate desiccation. Tolerant flowering plants grow up to about 3 m tall but are largely confined to highly xeric habitats or microhabitats (Alpert, 2000; Alpert and Oliver, 2002). Underlying these apparent morphological and ecological limits to desiccation tolerance may be inherent or phylogenetic limits to tolerance or physical or physiological constraints.

Understanding the limits to desiccation tolerance is likely to help us extend its frontiers. Perhaps the most exciting scientific frontier of the study of desiccation tolerance is how to induce or engineer tolerance in sensitive species (Crowe et al., 2005; Potts et al., 2005). Desiccation tolerance has already been induced in human blood cells, greatly enhancing their medical use. Because no crops tolerate desiccation, drought remains a major cause of famine, and engineering desiccation tolerance in crops might save many more lives.

The objective of this review is to consider what might limit the scope of desiccation tolerance in living things, whether these limits pose obstacles to extending the frontiers of tolerance through human intervention, and, if so, how these obstacles might be overcome. The review offers a definition of desiccation...
tolerance, takes up different categories of possible limits, and ends by considering their relationship to success in conferring tolerance upon new species. There have been a number of previous reviews of desiccation tolerance in individual taxa, and the review seeks to build upon these by citing them and more recent research papers.

**Defining Desiccation Tolerance**

“Desiccation tolerance,” as used here and in the other papers in this volume, is the ability to dry to equilibrium with air that is moderately to extremely dry and then regain normal function after rehydration. There are three key points to make about this definition. First, desiccation tolerance is not the same thing as drought tolerance. Instead, desiccation tolerance is one mechanism of drought tolerance. Drought is low water availability in the environment of an organism, whereas desiccation, as used here, is low water content in its cells. Many organisms tolerate drought by not desiccating, through mechanisms such as water storage in desert cacti or water synthesis in desert rodents. These organisms cannot desiccate without dying.

Second, desiccation tolerance here refers to complete desiccation, meaning complete air-dryness. In the literature on marine algae and animals (e.g., Hoffman and Harshman, 1999; Skene, 2004), desiccation tolerance has sometimes been used to mean the ability to survive drying below full or optimal water content, that is, partial desiccation. An important functional difference between complete and partial desiccation is that complete desiccation seems always to be accompanied by cessation of measurable metabolism, whereas studies of partial desiccation often focus on maintenance of metabolism (e.g., Danks, 2000). To specify tolerance of complete desiccation, zoologists coined the term, “anhydrobiosis” (e.g., Crowe et al., 1992). This is a synonym for “desiccation tolerance” as generally used in the literature on plants and as used here.

Third, a good quantitative definition of complete desiccation is probably drying to <0.1 g H₂O g⁻¹ dry mass (10% water content [WC]) or less. This is roughly equivalent to air-dryness at 50% relative humidity and 20°C and corresponds to a water potential of about −100 MPa (Gaff, 1997; Haranczyk et al., 1998; Proctor, 2003). For example, desiccation-sensitive seeds die before they dry to 20% WC, whereas tolerant seeds survive below 7% (Tweddle et al., 2003). Sensitive prokaryotes fail to survive drying to 30% WC (Billi and Potts, 2002). The threshold of 10% WC appears to have biological meaning, since it may correspond to the point at which there is no longer enough water to form a monolayer around macromolecules, stopping enzymatic reactions and thus metabolism (Billi and Potts, 2002).

It is not clear whether there is a continuum between desiccation tolerance and sensitivity. The best evidence for a continuum is in seeds (Sun and Liang, 2001; Song et al., 2003; Berjak and Pammenter, 2004). For instance, Tweddle et al. (2003) classed about 2% of some 8,000 species of plants as having seeds that were intermediate between being desiccation-tolerant and sensitive, based on their ability to survive drying below 20% but not below 10% WC. Some intertidal algae show intermediate tolerance, defined as surviving a water potential of <−15 MPa but not <−150 MPa (Abe et al., 2001; Burritt et al., 2002). Additional evidence comes from quantitative biochemical differences between tolerant and sensitive species, and from gradations in tolerance during development. Some of the mechanisms involved in desiccation tolerance by adult seed plants, such as synthesis of LEA proteins in response to dehydration, also occur to a lesser extent in sensitive species (Bartels and Salamini, 2001; Ranjanju and Bartels, 2002). Tolerance diminishes with age in some adult plants (Beckett, 2001; Vander Wijden et al., 2003) and animals (Ricci and Pagani, 1996). On the other hand, there appear to be no reports of adult plants or animals that survive drying to equilibrium with 80% relative humidity but not 50%. If there is not a complete gap between desiccation tolerance and sensitivity, there is certainly a strong modality, with many sensitive, some tolerant, and few intermediate species found so far.

**The Limits of Tolerance**

**Inherent limits**

A limit to a trait that appears to hold for all organisms might be called an “inherent limit,” especially if the limit is imposed by physics or some feature that seems essential to life. For example, if a molecule that all living things require and that none can synthesize de novo is destroyed below a certain water content, this could be an inherent limit to the minimum water content that living things can survive. Neither evolution nor engineering is likely to transgress such limits, and one might begin to look for them empirically by looking for records of tolerance.

The minimum water content or water potential to which desiccation-tolerance cells can survive drying is extremely low. Bacteria can recover from drying to 2% WC (Potts, 1994), and plants and animals from drying to equilibrium with a relative humidity of <1% (e.g., Pickup and Rothery, 1991). This seems likely to permit tolerant species to survive the most intense natural drought, and so not to limit their tolerance of drought.

The maximum length of time that desiccation-tolerance organisms can survive in the dry state is also very great. A seed of the sacred lotus, Nelumbo nucifera, was radiocarbon dated as being about 1,100 years old and successfully germinated (Shen-Miller et al., 1995). This seed was retrieved from an ancient lake bed; other records of tolerance come from specimens stored indoors. Mosses and liverworts have recovered after 20–25 years at air-dryness, and adult angiosperms and pteridophytes after 5 years (Alpert and Oliver, 2002). The cyanobacterium Nostoc commune shows no significant DNA damage after 13 years of...
being dry and can resume growth after 55 years in herbarium storage (Shirkey et al., 2003). Egg cysts of *Artemia* survive 15 years of desiccation (Clegg, 1967). Reports that nematodes can survive for 20–40 years may not be reliable, but rotifers and tardigrades can survive at least 9 years (Guidetti and Jonsson, 2002). It seems likely that some species from every major group with tolerant species can survive at least several years of desiccation. If survival under natural conditions is similar to survival in storage, this is not a limit to tolerance of natural drought almost anywhere on Earth, nor an obstacle to engineering tolerance.

Since drought is often accompanied by heat or cold, desiccation tolerance might not enable organisms to survive drought if they are sensitive to extreme temperatures. However, at least some desiccation-tolerant plants and animals can tolerate, not only heat and cold, but also high doses of radiation and poisons while desiccated (Alpert, 2000; Hoekstra, 2005). For example, desiccated tardigrades can survive treatments with X-rays and UV, and temperatures from near absolute zero to over 100°C (Jönsson and Bertolani, 2001). Nematodes, rotifers, and tardigrades can all survive fumigation with methyl bromide (Jönsson and Guidetti, 2001). High tolerance of radioactivity has led to the discovery of new desiccation-tolerant bacteria in radioactive work areas (Phillips et al., 2002b; Venkateswaran et al., 2003). The ability of many desiccation-tolerant animals to survive environmental extremes appears to exceed the extremes they ever encounter in nature, posing an interesting question as to the evolutionary origin of desiccation tolerance (Jönsson, 2003). In sum, the records of tolerance of drought and temperature by desiccation-tolerant organisms indicate that inherent limits to tolerance are not likely to pose an obstacle to the evolution or engineering of desiccation tolerance.

**Phylogenetic limits**

Although desiccation tolerance is known from many different clades, it is apparently absent from most phyla of animals, including chordates. If tolerance is a derived trait, then its evolution in clades of sensitive species might be limited by availability of genetic variation. If tolerance is a basal trait, then its re-evolution following loss within a clade might be similarly limited. Such “phylogenetic limits” to desiccation tolerance need not pose an obstacle to engineering tolerance in sensitive species, since it should be possible to introduce genes from tolerant species. Identifying phylogenetic limits is therefore a way of identifying opportunities to extend the frontiers of tolerance.

Two lines of evidence suggest that desiccation tolerance in plants is not phylogenetically limited. First, phylogenetic analyses indicate that tolerance in land plants is a basal trait (Oliver et al., 2000, 2005). Tolerance may have been lost in vegetative tissues in association with the evolution of internal water transport, but conserved in the spores and seeds of vascular plants. Independent phylogenetic analysis suggests that desiccation tolerance in seeds is a basal characteristic (Dickie and Prichard, 2002). Re-evolution of tolerance in adult plants, which appears to have occurred at least nine times (Oliver et al., 2000), may depend mainly on changes in gene expression since the genes necessary for tolerance in seeds or pollen are generally already present (Bartels and Salamini, 2001). The grass *Eragrostis nindensis*, whose seeds are tolerant, seedlings are sensitive, and adults are tolerant (Vander Wijlgen et al., 2003), might represent an intermediate in re-evolution of adult tolerance. One might thus be able to engineer tolerance in sensitive plants by manipulating regulatory switches for tolerance genes (Bartels and Salamini, 2001), although multiple changes in regulation are likely to be needed (Ramanjulu and Bartels, 2002).

The second line of evidence comes from comparisons of the genes associated with desiccation tolerance in different plants. Considerable homology between the structural genes contrasts with differences between the regulatory genes, suggesting that each re-evolution of adult tolerance has involved novel changes in the regulation of a similar set of structural genes (D. Bartels, personal communication). For instance, at least four novel regulatory genes occur in the desiccation-tolerant angiosperm *Craterostigma plantagineum* (Bernacchia and Furini, 2004). Comparison of phospholipases in *C. plantagineum* and *Arabidopsis thaliana*, which is desiccation-sensitive, suggests that homologous genes have been recruited to different purposes in the two species, to senescence and stomatal closure in *Arabidopsis* and to desiccation tolerance in *C. plantagineum* (Bartels and Salamini, 2001); evolution of tolerance may partly involve recruiting more existing genes to that purpose through regulation. This is not to suggest that the structural genes for tolerance are identical in all desiccation-tolerant plants. There are different routes to the synthesis of sucrose associated with desiccation tolerance in different species (Bartels and Mattar, 2002). Novel structural genes have been identified in individual species, such as XvPer1, which encodes a stress-inducible antioxidant enzyme in *Xerophyta viscosa* (Mowlra et al., 2002); the gene family CpPTP, which encodes proteins that may reversibly restructure chloroplasts during desiccation in *C. plantagineum* (Phillips et al., 2002a); and CpEdi-9, which encodes a hydrophilic protein in mature seeds and in response to dehydration in leaf phloem in *C. plantagineum* (Rodrigo et al., 2004).

Further support for the idea that evolution of desiccation tolerance in plants is not strongly limited by availability of genetic variation comes from the biogeography of tolerant angiosperms (Porembski and Barthlott, 2000). The majority are found largely on large rock outcrops, or inselbergs, in the tropics. Different continents have very different sets of species, consistent with evolution of tolerance in different groups of angiosperms subject to similar selective pressures. Biogeography does suggest that there may be some phylogenetic limitation to tolerance, since...
rock pools on inselbergs in western and in southeastern Africa both have tolerant bryophytes and lichens but only those in southeastern Africa have tolerant angiosperms (Krieger et al., 2000).

In contrast, it seems possible that the extent of desiccation tolerance may be phylogenetically limited in major clades of animals. Direct evidence is very limited, since there appear to be no phylogenetic analyses of tolerance in animals apart from a survey suggesting that tolerance is a basal characteristic in the class Bdelloidea of the rotifers (Ricci, 1998). There is also some remarkable counterevidence in the apparent homology of some genes associated with desiccation tolerance in different phyla of animals and even across animals, plants, and microbes. Named for their expression during induction of desiccation tolerance in maturing seeds, these Late Embryogenesis Abundant (LEA) genes encode a set of hydrophilic proteins that include dehydrins, which are produced in response to drying in tolerant plants. In animals, the products of LEA genes may act as molecular chaperones for DNA or counter physical stress during desiccation (Wise, 2003). In plants, they may increase the transition temperature and hydrogen bonding strength of sucrose glasses (Wolkers et al., 2001a), helping to inhibit membrane fusion, protein denaturation, and effects of free radicals (Oliver et al., 2001). LEA homologues have been found in nematodes and bacteria (Goyal et al., 2003; Browne et al., 2004; Wise and Tunncliffe, 2004), yeast (Garay-Arroyo et al., 2000; Sales et al., 2000), pollen (Wolkers et al., 2001a), bryophytes (Alpert and Oliver, 2002), roots and shoots of dicotyledonous plants (Schiller et al., 1997), and shoots of monocots (Bartels and Mattar, 2002).

Akin to phylogenetic limitation due to absence of genes for tolerance might be limits due to linkage between genes for tolerance and other genes, such that selection for traits that are not functionally linked to tolerance nevertheless counters selection for tolerance (e.g., Feldgarden et al., 2003). For example, genetic linkage between certain leaf traits and patterns of allocation in some plants appears to counter simultaneous adaptation of leaves and allocation to climate change (Etzler and Shaw, 2001). In Arabidopsis, correlation between water use efficiency and time required to reach flowering might seem to indicate a functional trade-off between drought tolerance and rate of reproduction but instead be at least partly due to fixation of genes that cause pleiotropy (Mckay et al., 2003). There seems to be no evidence so far for limits on tolerance due to genetic linkage in plants. However, lack of available genetic variation is one of several plausible explanations for the apparent complete absence of desiccation tolerance in most major clades of animals.

**Physiological constraints**

A limit to one trait that is imposed by the state of another trait is reasonably termed a “constraint,” given the original sense of “constrain,” to force to do something; and its literal sense, to tie together or compress by tying (OED, 1989). A physiological constraint on a biological unit may occur when it is controlled by the same physiological mechanism as another trait, since selection and regulation of the first trait may be limited by selection and regulation of the other. A prime example in animals is the regulation of multiple traits by endocrine systems (Ricklefs and Wikelski, 2002; Zera and Zhao, 2004). In plants, control of multiple traits by hormones can also impose physiological constraints (Farnsworth, 2004); tolerance of desiccation may be constrained by multiple effects of the hormone, abscisic acid or ABA.

ABA upregulates some aspects of desiccation tolerance in bryophytes (Beckett et al., 2000; Guschina et al., 2002; Zeng et al., 2002; Mayaba and Beckett, 2003), seeds (Wakui and Takahata, 2002), vegetative tissues of angiosperms (Bartels and Salamini, 2001; Bernacchia and Furini, 2004; Vicre et al., 2004), and possibly ferns (Pence, 2000). This has not been specifically tied to other effects of the hormone in tolerant species, but ABA tends in general to have effects that lead to slower growth as well as higher tolerance of stress by plants (Farnsworth, 2004) and selection for growth might counter selection for tolerance. For instance, a population of the desiccation-sensitive herb Impatiens capensis from a relatively wet habitat showed less response to ABA than a population from a drier habitat; this could reflect selection in the first population for avoidance of reduction in reproductive output due to ABA-mediated responses to cues for drought that are inappropriate in a wetter habitat (Heschel and Hausmann, 2001).

Cytokinins are a second important example of plant hormones with multiple effects (Farnsworth, 2004). No role has been established for cytokinins in desiccation tolerance, but levels change in association with desiccation in at least one tolerant species. As they dry, leaves of Craterostigmaholmii show a initial decrease in two cytokinins, zeatin and zeatin riboside, and then an increase below 20% relative water content (RWC: WC/WC at full turgor; Vicre et al., 2004). Levels return to normal after rehydration to 70% RWC. Understanding the physiological constraints governed by plant hormones could identify opportunities to engineer tolerance of desiccation and other stresses (Farnsworth, 2004; Kim et al., 2004). For instance, if control by ABA couples tolerance to slow growth, one might try to engineer release of tolerance from this control. Such release has already been engineered in transgenic callus tissue of C. plantagineum through constitutive expression of the regulatory gene CDT-I (Bartels and Salamini, 2001).

Another potential area of physiological constraint on tolerance may be regulation of senescence. Glutathione is oxidized during desiccation in lichens, and desiccation tolerance is correlated with the ability to reduce it again upon rehydration (Kranner, 2002). Correlation between levels of reduced glutathione and tolerance could be related to prevention of oxidation damage...
during desiccation. For example, plants of an intertidal red algae collected from higher in the intertidal zone and therefore subject to greater desiccation showed greater upregulation of enzymes required to regenerate glutathione and ascorbate during dehydration down to 40% WC and produced less hydrogen peroxide and lipid hydroperoxides (Burritt et al., 2002). However, a low ratio of reduced to oxidized glutathione can also cue apoptosis, suggesting that rapid reduction may also be needed to avoid prompting cell death (I. Kraner, personal communication).

Physical constraints

Physical traits that might constrain tolerance of desiccation include rigidity and size. Tolerant animals shrink as they desiccate, and it has been suggested that active contraction helps prevent mechanical damage during desiccation (e.g., Ricci et al., 2003). For instance, rotifers and tardigrades adopt distinctive, highly compact forms as they dry (Jönsson, 2001; Ricci et al., 2003), and coiling has been used as a criterion for non-lethal desiccation in nematodes (Treonis et al., 2000). Moreover, no desiccation-tolerant animals have rigid skeletons, at least not at the life stages where tolerance is present.

Desiccation-tolerant plants do have relatively rigid cell walls and even wood. Special wall morphologies may promote folding as cells dry and reduce mechanical stress during desiccation in pteridophytes (Thomson and Platt, 1997) and angiosperms (Farrant et al., 1999; Vander Willigen et al., 2004). Some angiosperms change their cell wall composition during drying (Vicre et al., 2004). In Craterostigma plantagineum, an increase in the transcription of an alpha-expansin cDNA during dehydration is associated with a rise in activity of expansin and in cell wall flexibility (Jones and McQueen-Mason, 2004). Shrinkage of plant cells during drying may be especially severe due to the presence of large, water-filled vacuoles, and some tolerant species appear to alleviate this by replacing large vacuoles with numerous small ones and filling them with non-aqueous compounds as cells dry (Thomson and Platt, 1997; Farrant, 2000).

Water transport through xylem imposes a second, related physical constraint on desiccation tolerance in vascular plants (e.g., Sherwin et al., 1998). Under most conditions and in all plants taller than about 3 m, water is pulled up rather than pushed up through files of dead cells in the xylem. When the negative pressure in the xylem exceeds that required to draw in an air bubble from a neighboring, air-filled conduit, cavitation results and interrupts water flow. In tolerant shrubs such as Myrothamnus flabellifolia, water columns must be regenerated after desiccation, and this is variously thought to occur via root pressure (Schneider et al., 2000) or capillary action (Sherwin et al., 1998), possibly modified by an unusual lining of lipids on the inner surface of the cell wall (Wagner and Farrant, 2000). Refilling may delay recovery from desiccation (Sherwin and Farrant, 1996) and is likely to be impossible at heights greater than 3 m. This may explain why there are no desiccation-tolerant trees, and a preponderance of tree species or possibly greater difficulty in refilling their special form of xylem may explain why there are no desiccation-tolerant adult gymnosperms, despite the presence of tolerance in gymnosperm pollen and seeds. Physical constraints on rehydration have been less explored in animals, but needs for water diffusion through cells might require all tolerant organisms to be small or thin (Potts, 2001).

Differentiation and multicellularity do not impose obvious constraints on desiccation tolerance in plants or animals. Desiccation-tolerant plants have a wide range of specialized forms. For instance, the floating and submerged leaves of Craterostigma intrepidus are specialized as sun and shade leaves (Woitke et al., 2004). Tolerance in the larva of the fly Polypedilum vanderplanki shows that tolerance is compatible with having a central nervous system; excised tissue without nerves also survives desiccation in this species, showing that the nervous system is not essential for tolerance (Watanabe et al., 2002).

Ecological limits

The likelihood that ability to tolerate intense or prolonged drought does not effectively limit desiccation tolerance in living things is corroborated by their occurrence in extreme habitats, such as in the hottest and coldest deserts and on bare, non-porous rock (Alpert, 2000; Alpert and Oliver, 2002). Tolerant cyanobacteria are probably the main source of primary productivity in some sand crusts in the Negev Desert (Harel et al., 2004); they can recover 50% of their normal photosystem II activity in as little as five minutes after rewetting. The Dry Valleys of Antarctica may be largely populated by desiccation-tolerant species, including nematodes in the soil (Treonis and Wall, 2005) and endolithic communities of lichens, cyanobacteria, and other bacteria (Hughes and Lawley, 2003). Granitic outcrops in Brazil sport films composed mainly of cyanobacteria plus cyanobacterial lichens (Buedel et al., 2002). Even a complete lack of liquid water may not exclude desiccation-tolerant species. For example, the alga Trentepohlia odorata can rehydrate and photosynthesize with water vapor (Ong et al., 1992).

On a finer scale, desiccation-tolerant plant species may nevertheless be excluded from the most xeric microsites within a habitat by inability to maintain a positive carbon balance over repeated cycles of desiccation and rehydration (Alpert, 1990, 2000). For instance, mosses at a site in the chaparral of southern California are much less abundant on the equator-facing than on the pole-facing surfaces of granitic boulders (Alpert, 1985). This is probably because they are unable to recoup respiratory losses of carbon during the night and during recovery from desiccation before desiccation in the sun ends photosynthesis again (Alpert and Oechel, 1985).

Although some aquatic plants and animals tolerate desiccation (e.g., Schill et al., 1997; Ricci, 1998),
Desiccation tolerance is negatively associated with occurrence in moist habitats. By far the most complete evidence for this is in seeds. Based on a survey of over 8,000 species of angiosperms, Tweddle et al. (2003) found that the proportion of species with desiccation-sensitive seeds tended to be higher in warmer and wetter habitats, and was highest (45%) among non-pioneer species in evergreen tropical forests. Within the genus *Coffea*, degree of desiccation tolerance in seeds decreases as number of dry months between dispersal and the start of the wet season decreases (Dussert et al., 2000).

Other comparisons between plants have mostly each involved just a few species but generally also found that those from wetter habitats were less tolerant. For instance, lower ability to maintain photosynthesis during desiccation was associated with higher ambient water availability in three species of Antarctic mosses (Robinson et al., 2000). Sensitivity to desiccation was associated with habitat wetness in other studies of mosses by Franks and Bergstrom (2000) and Seel et al. (1992), and in the common but not the rare mosses studied by Cleavitt (2002). Schipperges and Rydin (1998) reported no relationship between recovery from partial drying and microhabitat in six species of the moss genus *Sphagnum*, but none of these species tolerated complete desiccation. Microdistributions of eight epiphytic pteridophytes in a Mexican cloud forest correlated more closely with tolerance of desiccation and high light than with maximum performance (Hietz and Briones, 2001). Exposure to high UV or PAR while desiccated did not affect photosystem II in the xeric lichen *Xanthorea parietina*, but did have a negative effect on a lichen from more shaded habitats (Solhaug et al., 2003). Similarly, of three mosses, only the one from the most xeric habitats, *Tortula ruraliformis*, recovered photosynthetic capacity if desiccated in the light (Seel al., 1992).

Comparisons between animals are fewer and less consistent. Solomon et al. (1999) reported an association between habitat dryness and desiccation tolerance in three strains of the nematode *Steinernema feliae*. Gal et al. (2001) found higher transcription of glycogen synthase during dehydration of *S. feliae* from a temperate than from a semi-arid habitat, suggesting a shift away from production of trehalose, a sugar associated with tolerance, in the temperate strain. No subtidal marine tardigrades tolerate desiccation, whereas some of the intertidal (Clegg, 1967; Jönsson and Järemo, 2003), semi-aquatic freshwater (Jönsson and Bertolani, 2001), and terrestrial species do. Of two congeneric tardigrades from the upper intertidal, the one from exposed microsites was tolerant while the one from within barnacles was not (Grønågaard et al., 1990; cf., Jönsson and Järemo, 2003), and tardigrades in a population from a habitat with higher relative humidity and temperature were less tolerant than those in a population from a less humid, cooler habitat (Horikawa and Higashi, 2004). However, there was no difference in tolerance between populations of tardigrades from the two sites sampled by Jönsson et al. (2001), nor between populations of nematodes from Greece and the UK (Menti et al., 1997). Torrentera and Dodson (2004) noted differences in the phenology of populations of *Artemia* in hypersaline pools and salterns that differed in salinity, pH, and desiccation in Yucatan. In one of the most systematic comparisons of tolerance in a group of animals, Ricci (1998) noted that, of 15 rotifer species representing all four families of the class *Bbelloidea*, all three desiccation-sensitive species were aquatic.

Two possible explanations for the scarcity of desiccation-tolerant species in moist habitats are competitive exclusion by desiccation-sensitive species and selection against tolerance when water availability is high. Although there appear to be no experimental tests for association between tolerance and competitive ability, it may be that desiccation-tolerant “extremophiles” are actually competitively inferior “normifuges.” Work on mutants of *Arabidopsis* suggests that loss of desiccation tolerance in seeds may require changes in relatively few genes (Ooms et al., 1993). Derived desiccation sensitivity in seeds and rotifers from moist habitats could be due to lack of selection to maintain tolerance or to selection against it, if there is a trade off between tolerance and growth or reproduction.

**Frontiers of Tolerance**

The two main applications of research on desiccation tolerance in the past two decades have been attempts to induce tolerance in human cells for medical purposes and to engineer tolerance in crop plants to make them less vulnerable to drought. Researchers have also tried to engineer or induce tolerance in agronomic bacteria and in nematodes used for biological control. Knowing what limits desiccation tolerance in prokaryotes and nematodes could also help control pathogenetic species that are naturally desiccation-tolerant (Breeuwer et al., 2003). There has been considerable success in conferring tolerance on isolated membranes and enzymes and on single cells (Billi and Potts, 2002; Crowe et al., 2005; Potts et al., 2005), which seems to accord with what we know about the inherent and phylogenetic limits of tolerance. There has been almost no success in crop plants, suggesting the importance of physical or physiological constraints on tolerance in whole, multicellular organisms.

**Making single cells tolerate desiccation**

Desiccation tolerance in mammalian cells can be induced by treatment with trehalose, a sugar accumulated during drying in many desiccation-tolerant animals. Whereas fresh human blood platelets have a shelf life of only about five days, platelets freeze-dried in a solution of trehalose can be stored much longer and then rehydrated for use (Wolkers et al., 2001b, 2002). This technique specifically preserves membrane microdomains (Crowe et al., 2003). Gordon et al. (2001) incubated human mesenchymal stem cells in 50 mmol
trehalose and 3% glycerol, air-dried and stored them under vacuum, and express mailed them from San Diego to Baltimore, where they recovered normal morphology, lability, and regeneration capacity after rehydration. Incubation in a medium with a high trehalose concentration can make corneal epithelial cells desiccation-tolerant (Matsuo, 2001). Chen et al. (2001) introduced trehalose into mammalian cells with an engineered protein that formed pores in the plasma membrane; having about \(10^{10}\) molecules of trehalose per cell enabled cells to survive at 5% relative humidity and 20°C for weeks. There are now techniques to load human red blood cells with trehalose (Satpathy et al., 2004). Under optimal conditions, sugar alone may suffice to make mammalian cells tolerate desiccation (Crowe et al., 2002). Human cells can also tolerate desiccation in the absence of added sugars if dried slowly and stored under vacuum (Puhlev et al., 2001).

Desiccation tolerance can be induced in the bacteria _Escherichia coli_ and _Pseudomonas putida_ with either trehalose or hydroectoine (de Castro et al., 2000; Tunncliffe et al., 2001; Manzanera et al., 2002, 2004). Loading the nitrogen-fixing mutualist bacterium _Bradyrhizobium japonicum_ with trehalose by incubating it in the sugar during growth greatly improved its subsequent survival of desiccation, which is a major cause of failure of inoculation of leguminous crops with the bacterium in the field (Streeter, 2003).

There has also been some success in engineering desiccation tolerance in mammalian cells and bacteria. Guo et al. (2000) used a recombinant adenovirus vector to express the otsA and otsB genes of _Escherichia coli_, which encode enzymes that synthesize trehalose, in human primary fibroblasts and were able to maintain infected cells in the dry state for up to five days. However, engineering mouse cells to produce 80 mmol trehalose did not make them fully desiccation-tolerant (de Castro and Tunncliffe, 2000), even when extracellular trehalose was supplied (Tunncliffe et al., 2001). Moving the spsA gene of a cyanobacterium into _E. coli_ resulted in production of sucrose-6-phosphate synthetase and a 10,000-fold increase in survival of desiccation (Billi et al., 2000). It is also possible to transfer genes into naturally tolerant cyanobacteria (Billi et al., 2001), which might thereby become a desiccation-tolerant source of useful metabolites. Gene transfer may even have been significant in some natural origins of desiccation tolerance: the tolerant bacterium _Dienecoccus radiodurans_ appears to have acquired homologues of putative plant desiccation tolerance genes by horizontal transfer (Makarova et al., 2001).

The success in making single cells tolerate desiccation fits with knowledge of the limits of tolerance. There seem to be no significant inherent limits on desiccation tolerance and no physical or physiological constraints that apply to single, non-rigid prokaryote or animal cells. If phylogeny limits tolerance in many animals via lack of available genetic variation for tolerance, then introducing genes for tolerance might be expected to successfully overcome this limit.

**Making whole organisms tolerate desiccation**

It has so far been possible to increase tolerance of partial desiccation in multicellular animals and plants, but not to make them desiccation-tolerant. Treatment with cold can increase production of trehalose and tolerance in nematodes used for biological control (Grewal and Jagdale, 2002). Breeding can increase tolerance of partial desiccation to some extent in the entomopathogenic nematode _Heterorhabditis bacteriophora_ (Strauch et al., 2004). Introduction of trehalose biosynthetic genes into plants can increase their production of trehalose and their tolerance of various stresses but has not resulted in desiccation tolerance (Penna, 2003). For example, regulated overexpression of genes from _E. coli_ in rice plants increased their trehalose concentration 3–10 times (Garg et al., 2002). The plants showed relatively low photooxidation and high ability to accumulate nutrients under salt, drought, or cold stress but did not tolerate desiccation.

Lack of success in making whole organisms tolerate desiccation may suggest that the known limits that apply to whole, multicellular organisms set major obstacles to engineering tolerance in them. For example, physical constraints in both animals and plants and physiological constraints due to involvement of hormones in tolerance in plants are likely to require more than just the engineering of synthesis of sugars to confer tolerance. To reduce mechanical stress, animals may need to contract during desiccation and plants need to have more flexible cell walls. Tolerance is unlikely to be engineered in plants taller than 3 m due to the problem of refilling xylem. What we know about the natural limits to desiccation tolerance in living things offers encouragement for further efforts to artificially extend tolerance to single-celled organisms and to individual cells and tissues of multicellular ones. However, we may need to know more about the limits of desiccation tolerance before we can expect to extend it to desiccation-sensitive whole plants and metazoans.

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INTRODUCTION


