

Evolution of halophytes: multiple origins of salt tolerance in land plants

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This paper is part of an ongoing series: 'The Evolution of Plant Functions'.

Abstract. The evolution of salt tolerance is interesting for several reasons. First, since salt-tolerant plants (halophytes) employ several different mechanisms to deal with salt, the evolution of salt tolerance represents a fascinating case study in the evolution of a complex trait. Second, the diversity of mechanisms employed by halophytes, based on processes common to all plants, sheds light on the way that a plant's physiology can become adapted to deal with extreme conditions. Third, as the amount of salt-affected land increases around the globe, understanding the origins of the diversity of halophytes should provide a basis for the use of novel species in bioremediation and conservation. In this review we pose the question, how many times has salt tolerance evolved since the emergence of the land plants some 450–470 million years ago? We summarise the physiological mechanisms underlying salt-tolerance and provide an overview of the number and diversity of salt-tolerant terrestrial angiosperms (defined as plants that survive to complete their life cycle in at least 200 mM salt). We consider the evolution of halophytes using information from fossils and phylogenies. Finally, we discuss the potential for halophytes to contribute to agriculture and land management and ask why, when there are naturally occurring halophytes, it is proving to be difficult to breed salt-tolerant crops.

Additional keywords: phylogeny, saline agriculture, salinity.

The freshwater origin of land plants

Water relations

All organisms are faced with balancing their water relations with those of their environment. Marine photosynthetic organisms achieve this balance at the water potential of seawater, about -2.3 MPa (Harvey 1966), using a mixture of ions and organic compounds (Bisson and Kirst 1995; Iwamoto and Shiraiwa 2005). Terrestrial plants must also adjust their water potential to be at least as low as that of the soil in which they are growing. For plants living in saline environments, the Na^+ concentration can range from ~ 100 to 2380 mM Na (Flowers 1985), equivalent to a water potential range from -0.5 to -11 MPa. This means that plants growing in saline soils must adjust osmotically to water potentials that are commonly in the range -2 to -3 MPa; they do this, as do marine plants, by accumulating a mixture of ions and organic solutes (e.g. Flowers *et al.* 1977). For all terrestrial plants, however, whether living in a saline or non-saline environment, the major factor affecting their water relations is the large difference in concentration of water vapour between their substomatal pores and the atmosphere – the driving force for the movement of water

through the soil–plant–atmosphere continuum (even at 90% RH the atmospheric water potential is -14.2 MPa). The consequent flux of water through the plant carries essential nutrients to the shoots, but in a saline environment, any dissolved solutes from the soil that enter the transpiration stream are also carried to the leaves where they must be accommodated, re-circulated or excreted.

The first land plants

Before the evolution of embryophytes (terrestrial plants that are not algae; *viz.* bryophytes, pteridophytes and spermatophytes), green plants were composed of the Chlorophyta and Streptophyta, which split ~ 1000 million years ago (Becker and Marin 2009). Palaeontological and molecular evidence suggests embryophytes arose from the Streptophyta rather than the Chlorophyta; from charophycean green algae (Graham 1993; Kenrick and Crane 1997; Raven and Edwards 2001; Waters 2003) in the Ordovician period, some 450 million years ago. This date is supported by molecular data (Sanderson 2003), although the transition to terrestrial habitats happened on several occasions (Lewis and McCourt 2004). While there is some uncertainty

concerning the origins of the embryophytes, with support for the Charales, Coleochaetales and the Zygnematales (Becker and Marin 2009) being sister to the embryophytes, recent evidence favours the Charales (Becker and Marin 2009).

The first records of land plants are microfossils consisting of spores and plant parts, which appeared in the mid-Ordovician (~470 million years ago); they are found through to the Early Silurian period (Wellman and Gray 2000). The earliest clearly recognisable land plants date from ~40 million years later, in the Early Late Silurian (Wellman and Gray 2000), in which plants such as *Cooksonia* can be seen in the fossil record (Graham 1993). The origin of these early embryophytes is crucial to the origin of salt tolerance in land plants as it could have evolved from species that lived in fresh or salt water: by the time the first transition of plants to a terrestrial environment occurred, the seas were saline (Railsback *et al.* 1990), even if a little less so (26–29 g per 1000 g seawater) than today's oceans (30–40 g per 1000 g seawater).

Did terrestrial plants derive from charophycean green algae living in fresh or salt water? Since Characean algae occupied both freshwater and marine habitats in the mid-Paleozoic (Kenrick and Crane 1997), early embryophytes could have arisen from marine or freshwater species. Any marine organism making the transition to the land on the edge of a saline pool would have to cope not only with water loss from its cells to the atmosphere, but also with hyper-saline conditions, which would have occurred as water evaporated from the pool. If, however, the first land plants evolved at the edges of freshwater pools (Raven and Edwards 2001), then land plants would have arisen from an organism (or organisms) that had adapted to living in fresh water.

A particularly important adaptation to freshwater life is the development of systems for acquiring nutrients from the low concentrations of minerals present in fresh water rather than the high concentrations of the nutrient rich (eutrophic) marine environment (Rodríguez-Navarro and Rubio 2006). This ability would be equally important for growth on land, where plants need to acquire nutrients in a nutrient poor (oligotrophic) environment. Any such adaptation to oligotrophy might have left intact those systems required for osmotic adjustment and which are necessary for growth in seawater or in fluctuating salinity. If embryophytes originated from freshwater algae, then, as suggested by Becker and Marin (2009), the streptophytes were 'physiologically pre-adapted to terrestrial existence by their primary freshwater lifestyle in contrast to their marine sister lineage, i.e. Chlorophyta' (perhaps because their adaptation to an oligotrophic lifestyle resulted in evolution of specific mechanisms for dealing with fluctuating ion concentration in the environment; see also *Ion compartmentation* below). Analysis of fossils from Rhynie in Aberdeenshire in Scotland suggests that by the Early Devonian, ~400 million years ago, plants were drought and probably salt tolerant (Channing and Edwards 2009). The question, then, is how many times salt tolerance has evolved since the emergence of the land plants some 450–470 million years ago.

Physiology of salt tolerance

Although there are many aspects of the physiology of salt tolerance that are yet to be understood, it is clear that the trait is complex in that, at a minimum, it requires the combination of

several different traits: the accumulation and compartmentation of ions for osmotic adjustment; the synthesis of compatible solutes; the ability to accumulate essential nutrients (particularly K) in the presence of high concentrations of the ions generating salinity (Na); the ability to limit the entry of these saline ions into the transpiration stream; and the ability to continue to regulate transpiration in the presence of high concentrations of Na⁺ and Cl⁻ (Flowers and Colmer 2008).

Commonality of traits

All plants, not just halophytes, have the main characteristics necessary for salt tolerance – the ability to acquire ions that are then compartmentalised in vacuoles (fundamental to turgor generation in plants and algae) and discrimination in favour of K over Na. Given that the physiological foundations of salt tolerance are found in all plants, it is not surprising that plant species show a continuum of salt tolerance with glycophytes such as chickpea (Flowers *et al.* 2010) at one extreme (where just 25 mM NaCl can be toxic) and halophytes (which can tolerate salt concentrations as high as 500–1000 mM) at the other. Furthermore, salt tolerance is often part of a multi-faceted syndrome that may include, for example, tolerance of flooding (Colmer and Voesenek 2009) and drought. Indeed, physiological responses to salinity are often similar to responses to other environmental stresses (Munns and Tester 2008) and may rely on common stress-tolerance pathways (Tuteja 2007).

Ion compartmentation

Most commonly, the ions dominating saline environments are those of Na and Cl, as they predominate in seawaters: halophytes use these ions for most of their osmotic adjustment (Yeo 1983). It is clear, however, that in the process of ion accumulation for osmotic adjustment, some ions can 'leak' into the transpiration stream (Yeo *et al.* 1987) and that such leaks must be minimised if the aerial parts are not to be swamped by ions (Munns 2005). Consequently, tight regulation of ion transport from roots to shoots is vital for halophytes. Sufficient Na and Cl must be accumulated to achieve osmotic adjustment but excess must be avoided as both ions are toxic at the concentrations required for osmotic adjustment. In practice, Na⁺ and Cl⁻ are largely compartmentalised in vacuoles in halophytes (Flowers *et al.* 1977, 1986). A range of metabolically inert organic compounds is also present and utilised to adjust the osmotic potential of the cytoplasm (Flowers and Colmer 2008). In some species, excess ions are secreted through glands that have evolved on the surface of the aerial parts. Recirculation of ions from shoots back to the roots is unlikely to be an important aspect of salt tolerance as the ions would have to be carried in the symplasm of the phloem where they would be toxic (Flowers *et al.* 1986; Munns and Tester 2008). Furthermore, phloem transport delivers solutes to growing regions where ions would cause metabolic damage unless re-exported to the environment. The latter would not remove ions from the rhizosphere. So, ions reaching the leaves must be accommodated in vacuoles in the leaf cells, a process that involves the transport of ions across both plasma and vacuolar membranes.

Land plants, including mosses and ferns, regulate their internal Na⁺ concentrations through the control of influx and efflux.

Within the eukaryotes, Na^+ efflux is mediated by Na^+/H^+ antiporters and Na^+ -pump ATPases, of which there are two types: the Na^+/K^+ -ATPases of animal cells and the fungal Na^+ -ATPases. Higher plants do not appear to express Na^+ -ATPases (Garcia-deblas *et al.* 2001) meaning that they must rely on Na^+/H^+ antiporters to transport Na^+ out of their cytoplasm, whether it be into the apoplast or the vacuoles. Benito and Rodriguez-Navarro (2003) argued that the absence of the Na^+ -ATPases in higher plants was evidence for their evolution in non-saline oligotrophic environments where Na^+ concentrations were low and there was no need for the system(s) than mediated extensive Na^+ efflux. This would presumably have occurred during the early development of land organisms in non-saline environments (Horodyski and Knauth 1994) or through the loss of such a system(s) following the migration of marine organisms to freshwater. Interestingly, the moss *Physcomitrella patens* (Bryopsida) expresses two ATPases similar to fungal ENA- Na^+ -ATPases (Benito and Rodriguez-Navarro 2003; Lunde *et al.* 2007). The presence of the Na^+ -ATPases in a moss suggests these enzymes were lost during early evolution of land plants (Benito and Rodriguez-Navarro 2003), which then rely on the Na^+/H^+ antiporters (also present in *P. patens*, Benito and Rodriguez-Navarro 2003) to regulate cytosolic Na^+ . The question of how plants living in seawater achieve this function remains. The Na^+/H^+ antiporters in plants are electroneutral (Munns and Tester 2008), which means they would not facilitate Na^+ efflux at the alkaline pH values of seawater (Benito and Rodriguez-Navarro 2003). However, seagrasses do presumably efflux Na^+ but how their Na^+/H^+ antiporters function in this respect is unclear (Garcia-deblas *et al.* 2007; Touchette 2007). The same question can be posed for halophytes, although it is possible that the rhizosphere pH could be lower than that of seawater; the operation of Na -efflux through antiporters has been recently questioned (Britto and Kronzucker 2009).

K/Na selectivity

The selectivity of halophytes for K over Na varies between families of flowering plants (Flowers *et al.* 1986). Net selectivity (net $S_{\text{K}:\text{Na}}$), calculated as the ratio of K concentration in the plant to that in the medium divided by the ratio of Na concentration in the plant to that in the medium, ranges between average values of 9 and 60 (Flowers and Colmer 2008) with an overall mean of 19; it is only in the Poales that net $S_{\text{K}:\text{Na}}$ values of the order of 60 are found. Within the monocots (Fig. 1), there are three orders with halophytes, but no data are available for the net $S_{\text{K}:\text{Na}}$ values of species within the Arecales. In the Alismatales, the average net $S_{\text{K}:\text{Na}}$ (across just three species) is 16 (range 10 to 22), suggesting that high selectivity has evolved only in the Poales (for halophytes within this order, average selectivities of are 58 in the Juncaginaceae (two species) and 60 in the Poaceae (nine species)). There is too little data to analyse the net $S_{\text{K}:\text{Na}}$ values within the dicots, but the average value is 11 compared with 60 in the Poales (Flowers and Colmer 2008).

Salt glands

Glandular structures are not uncommon on plants; they can secrete a range of organic compounds (Wagner 1991;

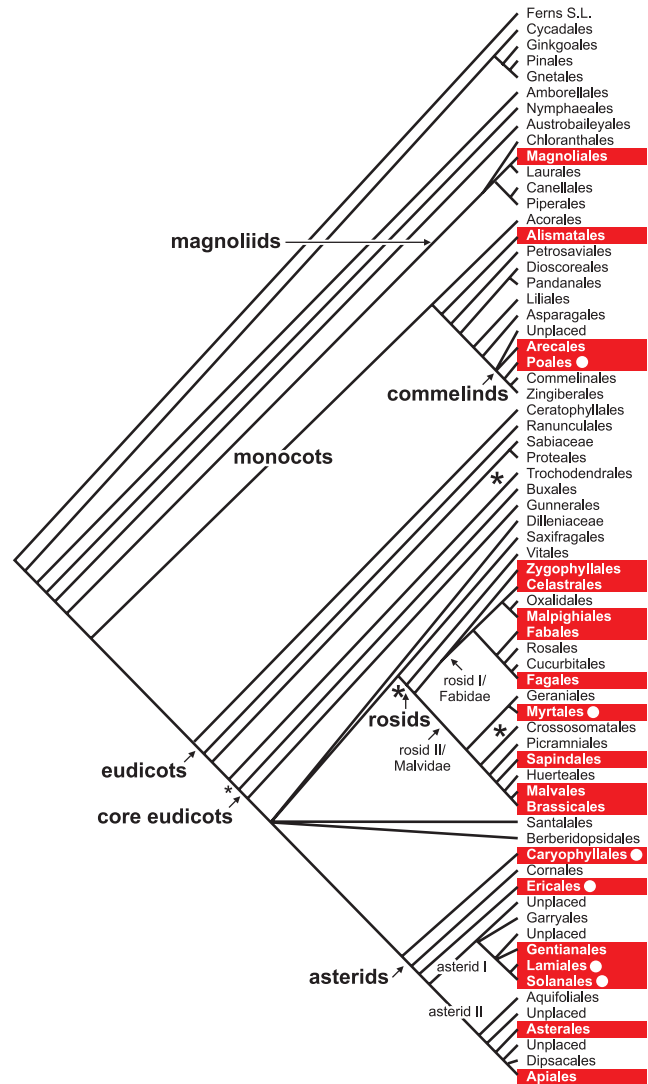


Fig. 1. The distribution of halophytes in the main tree by Stevens (2001), based on species listed by Aronson (1989) that survive at least 200 mM NaCl. Those orders containing halophytes are boxed in red, and the white circle indicates some species within the order have salt glands. * Indicates a node with rather weak support according to Stevens (2001).

Wagner *et al.* 2004). However, the ability to secrete salt appears to have evolved less frequently than salt tolerance. Salt glands, epidermal appendages of one to a few cells that secrete salt to the exterior of a plant (Thomson *et al.* 1988) have been described in just a few orders of flowering plants – the Poales (e.g. in *Aeluropus littoralis* and *Chloris gayana*), Myrtales (e.g. the mangrove *Laguncularia racemosa*), Caryophyllales (e.g. *Mesembryanthemum crystallinum* and the saltbush *Atriplex halimus*), Lamiales (e.g. the mangroves *Avicennia marina* and *Avicennia germinans*) and the Solanales (e.g. *Cressa cretica*). Their distribution across the orders of flowering plants suggests at least three origins, although there may have been more independent origins within orders (see for example within the Caryophyllales, Fig. 2b). Whether salt glands evolved from

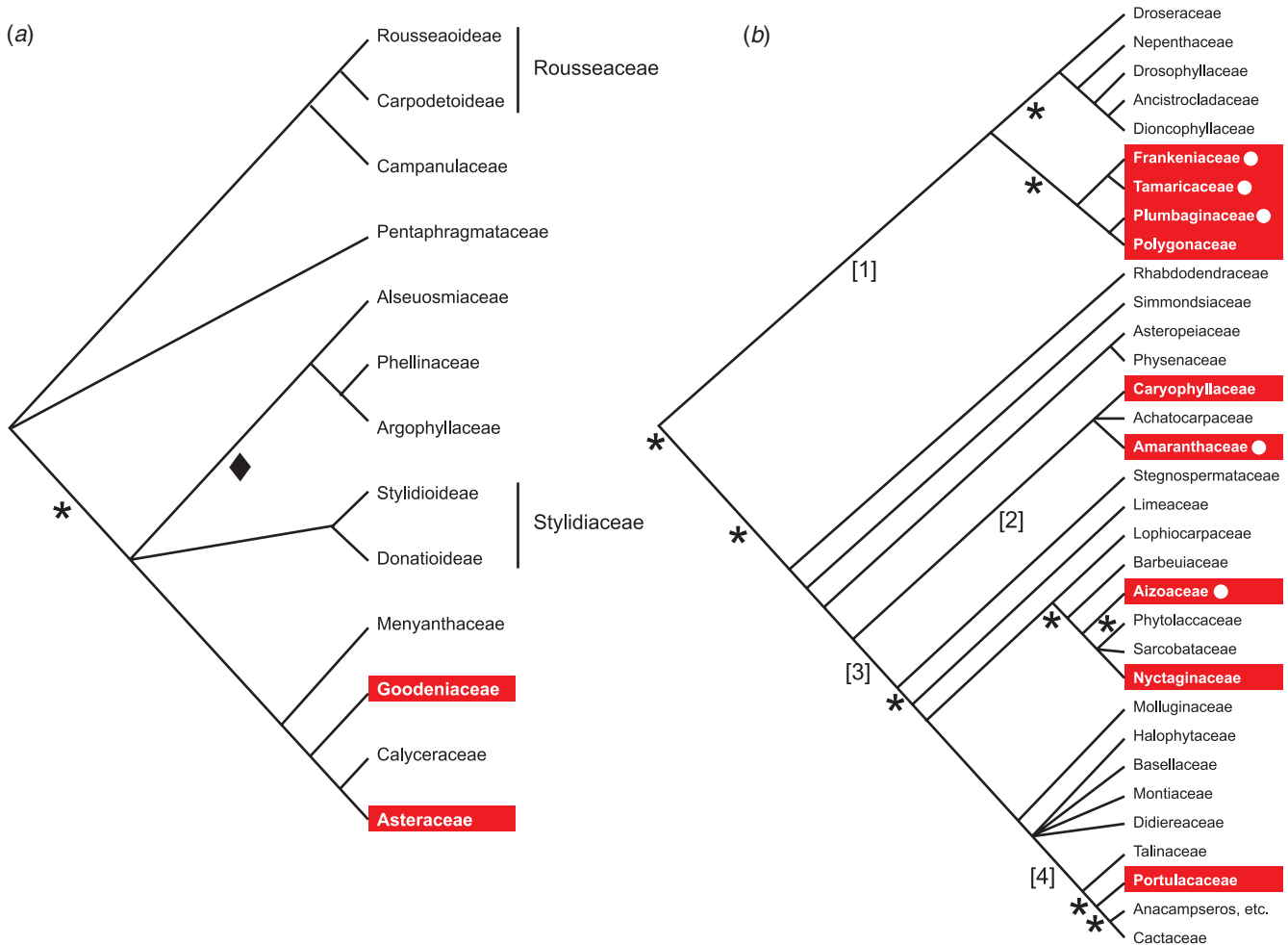


Fig. 2. The distribution of halophytes in phylogenetic trees by Stevens (2001), based on species listed by Aronson (1989); (a) Asterales and (b) Caryophyllales. Those orders containing halophytes are boxed in red, and the white circle indicates some species within the order have salt glands. For (a) * denotes branches with 100% posterior probability and ◆ denotes branches with 70–80% jackknife support: all other branches have >80% support (according to Stevens 2001). In (b) * denotes nodes with 50–80% bootstrap support; unmarked branches have >80% support; [1] and [3] have 50–80% bootstrap support in matK tree only and [2] is not recognised at all in matK tree only (according to Stevens 2001).

glands that originally performed some other function is unclear, but it is difficult, at least in the Poaceae, to get glandular hairs on non-halophytes (such as *Zea mays* L.) to secrete salt (Ramadan and Flowers 2004).

Compatible solutes

The compatible solutes synthesised by halophytes range from quaternary ammonium compounds through sulfonium analogues to proline and sugar alcohols: most are widely distributed through the orders of flowering plants, reflecting both phylogeny and functional needs (Flowers and Colmer 2008). There is no clear pattern of particular solutes being exclusive to particular orders, other than of sorbitol to the Plantaginaceae, so that it appears that although the ability to synthesise and compartmentalise a compatible solute is a requirement in halophytes, the nature of that solute depends on the habitat and the availability of N. Compatible solutes comprise a small proportion of the total solutes accumulated by halophytes (Yeo 1983).

How many halophytes are there?

As a trait, salt tolerance has been recognised in plants for more than 200 years (Flowers *et al.* 1986) and there have been several definitions of what constitutes a ‘halophyte’. Angiosperm species show a wide range of levels of salt tolerance from inhibition of growth at low salt concentrations (e.g. sensitive chickpea genotypes dying in 25 mM NaCl, Flowers *et al.* 2010) to tolerance of salt concentrations as high or higher than seawater (e.g. the halophyte *Arthrocnemum macrostachyum* survives in up to 1000 mM NaCl, Khan *et al.* 2005). Any definition of a ‘halophyte’ must include a threshold value of salt concentration and the number of halophytes clearly depends on the salt concentration used in that definition. By setting a dividing line between halophytes and glycophytes (salt sensitive plants) at a salt concentration of ~80 mM NaCl (a conductivity in the soil solution of 7.8 dS m⁻¹), Aronson (1989) listed ~1550 salt-tolerant plants. Using the same definition, Menzel and Lieth (2003) recorded over 2600 species. These comprehensive lists

define salt tolerant plants using a salt concentration that is much lower than that present in seawaters (an average of ~480 mM Na⁺ and 580 mM Cl⁻). Since there are species that have evolved tolerance to seawater salinities, we have chosen to use a salt concentration of '~200 mM salt' as our defining limit (as did Flowers and Colmer 2008): precision is not required as salt concentrations in natural habitats fluctuate with evaporation and precipitation of water. Applying the definition of 'plants that survive to complete their life cycle in at least 200 mM salt' to Aronson's (1989) database generates a list of some 350 species, which we call 'halophytes' (whether this group of the most salt tolerant of plant species should be given a separate name, such as 'euhalophyte', is an issue that could be further debated). Our halophytes are not uniformly distributed across the taxa of flowering plants listed by Stevens (2001). The orders with the greatest number of halophytes (Table 1) are the Caryophyllales (which includes those species that were formerly in the Chenopodiaceae such as the saltbushes in the genus *Atriplex*, genera with reduced leaves such *Salicornia* and *Tectomia* and succulent members of the genus *Suaeda*) and the Alismatales (which includes marine flowering plants belonging to genera such as *Zostera* and *Thalassia*). Although our list of halophytes is not comprehensive as the limits of tolerance for many species are not known, our analysis suggests that halophytes are not more than ~0.25% of the known species of angiosperms.

Salt tolerance in bryophytes and pteridophytes

Halophytes are uncommon among the higher plants and this situation is also true for bryophytes and pteridophytes. There are some mosses that occur in areas that are inundated by tides (Table 2); for example, four species in Northern England (Adam 1976) and five species in Nova Scotia, one of which has been shown to survive immersion in seawater for 24 h (Garbary *et al.* 2008). Aronson (1989) lists only one genus of a salt resistant fern. This low frequency of salt tolerance in bryophytes and pteridophytes indicates that they also have rarely evolved the ability to tolerate salt concentrations approaching those of seawater, although some are able to grow in lower concentrations (e.g. *Physcomitrella patens* in 100 mM NaCl, see Lunde *et al.* 2007).

Repeated evolution of salt-tolerance in angiosperms

In an earlier analysis based on the salt tolerance criterion used by Aronson (1989) and a phylogeny constructed by Takhtajan (1969), Flowers *et al.* (1977) concluded that salt tolerance was widely distributed amongst the families of flowering plants and suggested a polyphyletic origin, something more recently established for other complex traits such as C₄ photosynthesis (Sage 2004) and crassulacean acid metabolism (Crayn *et al.* 2004). In Fig. 1, we have plotted the distribution of clades containing halophytes (as defined above) on an order-level phylogeny of angiosperms based on the main tree published

Table 1. The distribution of halophytes amongst orders of flowering plants

The estimates of species frequency are approximate due to the inexact numbers of species within families, the change in taxonomy that has occurred since the species were listed by Aronson (1989) and as the limits of tolerance for many species are not known. Examples of halophytes and suggestions for model species can be found in Flowers and Colmer (2008)

Order	Number of families	Number of families with halophytes	% Families with halophytes within the order	Number of halophytic species	% of all halophytic species (345) ^A
Caryophyllales	34	9	26	74	21.4
Alismatales	14	7	50	61	17.7
Malpighiales	40	4	10	35	10.1
Poales	16	4	25	28	8.1
Lamiales	25	5	20	24	7.0
Myrtales	12	4	33	22	6.4
Fabales	4	1	25	21	6.1
Malvales	10	1	10	18	5.2
Ericales	25	6	24	13	3.8
Arecales	1	1	100	11	3.2
Gentianales	5	4	80	9	2.6
Sapindales	9	3	33	8	2.3
Asterales	11	2	18	8	2.3
Zygophyllales	2	1	50	3	0.9
Apiales	7	1	14	3	0.9
Magnoliales	6	1	17	2	0.6
Solanales	5	1	20	2	0.6
Celastrales	4	1	25	1	0.3
Fagales	8	1	13	1	0.3
Brassicales	18	1	6	1	0.3
Totals	256	58	–	345	100

^A345 is the total number of halophytes included in this analysis.

Table 2. Salt tolerant mosses and ferns

Species names	Location	Reference
<i>Mosses</i>		
<i>Pottia heimi</i> , <i>Amblystegium serpens</i> , <i>Eurhynchium praelongum</i> <i>Cratoneuron filicinium</i> and <i>C. stellatum</i> ^A	Northern England	Adam (1976)
<i>Campylium stellatum</i> , <i>Bryum capillare</i> , <i>Didymodon rigidulus</i> , <i>Mnium hornum</i> and <i>Am. serpens</i>	Nova Scotia	Garbary <i>et al.</i> (2008)
<i>Ferns</i>		
<i>Acrostichum aureum</i> , <i>Ar. danaeifolium</i> and <i>Ar. Speciosum</i>	Tropics	Aronson (1989)

^A*Cratoneuron stellatum* was shown to survive immersion in seawater for 24 h (Garbary *et al.* 2008).

by Stevens (Stevens 2001). From this order-level tree we can see that salt tolerance seems to have evolved multiple times.

We cannot determine the exact number of origins of salt tolerance from this phylogeny for several reasons. First, without information on the number of halophytes within each order, and their relationships to each other, we cannot know whether each family containing halophytes represents a single origin or multiple origins (see below). Second, if a clade contains both halophytes and non-halophytes, we cannot be sure whether salt tolerance was gained independently in multiple lineages, or whether the condition is ancestral to the clade and then lost in some families. Third, the evidence for the presence of halophytes in some orders is based on incomplete data (e.g. there are just two species reported as able to grow in seawater in the Magnoliales (Annonaceae) and without any physiological data). In most orders that contain families with halophytes, the percentage of halophytes is 1% or less (Table 1), which makes it unlikely that the condition is ancestral and the non-halophytes lost salt-tolerance. Therefore it seems likely that virtually each of the red boxes in Fig. 1 represents at least one independent origin of salt tolerance.

Many of the orders marked in Fig. 1 may contain multiple origins of halophytes. For example, within the Asterales (Fig. 2a), we have recorded eight halophytes in two families (seven in the Asteraceae and one in the Goodeniaceae), but the remaining families do not have any halophytes. It is parsimonious to assume that at least two lineages within the Asterales evolved salt tolerance independently. Examining the presences of particular traits associated with salt tolerance may also reveal some interesting evolutionary patterns. For example, five of the nine salt-tolerant lineages within the Caryophyllales use salt glands to excrete excess salt from the leaves. This illustrates how even related lineages may develop different strategies for dealing with environmental salt. The distribution of salt glands within the phylogeny (Fig. 2b) suggests that even within this single clade of flowering plants, a key salt tolerance mechanism has evolved independently several times.

It also seems likely that the distribution of halophyte-containing families is not random over the phylogeny, with

some clades containing more putative origins of salt tolerance than others. If true, this would seem to offer support for the idea that some clades are more likely to give rise to halophytes than others. It may be profitable to look at the physiology of members of those clades to see if they share certain characteristics that make adapting to environmental salt easier than in other clades. Mapping key components of salt tolerance on phylogenies may help to reveal whether some lineages are predisposed to evolving particular solutions to salinity, whether some traits that confer salt tolerance are more easily acquired than others, or even whether the component traits of salt tolerance are usually acquired in a particular order. Looking at the patterns of origination within families will be important for answering the questions of whether there can be an ancestral condition that favours the evolution of salt tolerance.

Importance of halophytes to agriculture and land management

Salinity is an expanding problem. More than 800 million ha of land is salt-affected, which is over 6% of the world's land area (Flowers and Yeo 1995). Salt-affected land is increasing worldwide through vegetation clearance and irrigation, both of which raise the watertable bringing dissolved salts to the surface. It is estimated that up to half of irrigation schemes worldwide are affected by salinity (Flowers and Yeo 1995). Although irrigated land is a relatively small proportion of the total global area of food production, it produces a third of the food (Munns and Tester 2008). Salt stress has been identified as one of the most serious environmental factors limiting the productivity of crop plants (Flowers and Yeo 1995; Barkla *et al.* 1999), with a huge impact on agricultural productivity. The global annual cost of salt-affected land is likely to be well over US\$12 billion (Qadir *et al.* 2008). Future agricultural production will rely increasingly on our ability to grow food and fibre plants in salt-affected land (Rozema and Flowers 2008; Qadir *et al.* 2008).

Breeding for salt tolerance

Although it is abundantly clear that, at some level, salinity reduces the growth of plants, the reason is generally not clear. Both Na⁺ and Cl⁻ can reach toxic concentrations if not compartmentalised in vacuoles, but within the complexity of the whole plant, the exact cause of any damage is generally unknown. However, the consequences are that salinity reduces plant growth and enhances senescence of mature leaves, resulting in a reduction in functional leaf area: in crop plants, this decreases yield (Munns *et al.* 2006; Munns and Tester 2008). There is, therefore, a great incentive to increase the salt tolerance of crop plants to allow greater yields in salt-affected soils. However, breeding of salt-tolerant crops is hampered by the complexity of salt tolerance as seen in halophytes (see above). So, despite a large amount of effort that has been directed towards increasing the salt tolerance of crops, ranging from wide-crossing to transgenics, few productive salt-tolerant varieties have been produced (Flowers and Yeo 1995; Flowers 2004; Flowers and Flowers 2005; Witcombe *et al.* 2008). The realisation that halophytes have evolved several times should encourage attempts to generate new salt-tolerant crops and may assist plant breeders in several ways. First, it may illuminate specific traits associated with salt tolerance

that may be selected in established crop plants (e.g. Huang *et al.* 2008); second, it may lead to the domestication of naturally salt tolerant species as crop plants.

Halophytes as crops

Naturally salt-tolerant species are now being promoted in agriculture, particularly to provide forage, medicinal plants, aromatic plants (Qadir *et al.* 2008) and for forestry (Marcar and Crawford 2004). For example, Barrett-Lennard (2002) identified 26 salt-tolerant plant species capable of producing products (or services) of value to agriculture in Australia. Examples of useful halophytes include the potential oil-seed crops *Kosteletzkya virginica* (e.g. Ruan *et al.* 2008); *Salvadora persica* (e.g. Reddy *et al.* 2008); *Salicornia bigelovii* (Glenn *et al.* 1991) and *Batis maritima* (Marcone 2003); fodder crops such as *Atriplex* spp. (e.g. El Shaer 2003), *Distichlis palmeri* (e.g. Masters *et al.* 2007) and biofuels (Y. Waisel, pers. comm.; Qadir *et al.* 2008). Growing salt-tolerant biofuel crops on marginal agricultural land would help to counter concerns that the biofuel industry reduces the amount of land available for food production (Qadir *et al.* 2008). At the extreme, plants that can grow productively at very high salt levels could be irrigated with brackish water or seawater (Rozema and Flowers 2008). Although plants that put resources (c.f. Yeo 1983) into developing salt-tolerance mechanisms (e.g. the production of compatible solutes to maintain osmotic balance is an energetic cost) may do so at the expense of other functions, many halophytes show optimal growth in saline conditions (Flowers and Colmer 2008) and salt marshes have high productivity (Colmer and Flowers 2008; Flowers and Colmer 2008). The fact that dicolytedonous halophytes can grow at similar rates to glycophytes (see Flowers and Colmer 2008 for a more detailed discussion) suggests that salt tolerance *per se* will not limit productivity. Here the contrast with drought tolerance is stark: without water plants do not grow, but may survive; with salt water, some plants can grow well.

Apart from direct use as crops, we may increasingly need to rely on halophytes for revegetation and remediation of salt-affected land. Over the last 200 years, industrialisation in Europe and elsewhere has led to an enormous increase of production, use and release of traces of heavy metals into the environment. A large portion of these toxic materials, including Cd, Cu, Pb and Zn, accumulate in sediments, including the soils of tidal marshes. Recent studies showed that some seagrasses and salt marsh plants are capable of extracting heavy metals from sediments and accumulating them in belowground or aboveground tissues (Weis and Weis 2004; Cambrolle *et al.* 2008; Lewis and Devereux 2009). The processes and potential application of these aquatic halophytes merits much greater research and development. Growing salt-tolerant plants, including species of *Kochia*, *Bassia*, *Cynodon*, *Medicago*, *Portulaca*, *Sesbania*, and *Brachiaria*, may also improve other soil properties, such as increasing water conductance or increasing soil fertility (Qadir *et al.* 2008). Halophytes may also lower the watertable, thereby allowing growth of salt-sensitive species in salt-affected land (Barrett-Lennard 2002).

Finally, conservation and land management in salt-affected regions will benefit from improved knowledge of the salt

tolerance of local species. Halophytes are being targeted for 'ecosystem engineering' projects, such as stabilising wetlands to increase the success of revegetation programs (Fogel *et al.* 2004). Halophytes are also being used to develop sustainable agricultural practise, for example, using indigenous halophytes to restore overgrazed pasture and to reduce reliance on irrigation (Peacock *et al.* 2003). Salt-tolerant species are increasingly being considered for landscape remediation. For example, well planned revegetation can reduce both the *in situ* salt concentration at mine sites and also reduce the movement of salts from the mine sites in runoff (Carroll and Tucker 2000). However, care must be taken in introducing species for bioremediation as they may become an environmental problem in their own right. For example, *Kochia* planted for forage and bioremediation in Western Australia in 1990 soon spread and was declared a weed in 1992 (Qadir *et al.* 2008).

The halophyte paradox

In this review, we have shown that salt tolerance has evolved many different times in angiosperms and in different ways. We have also noted that, despite increasing salinisation of agricultural land, few salt-tolerant crops have been produced. This raises an important paradox: if all plants contain the basic physiological mechanisms on which salt tolerance is based and if halophytes have evolved in many different lineages using a variety of adaptations, then why is it so difficult to breed salt-tolerant crops?

The reasons for the slow progress in changing the salt tolerance of plants were discussed by Flowers and Yeo (1995), who argued that this was because salinity had not become a problem of sufficient priority that the necessary resource was being applied to its solution. Moreover, they also concluded that treating salinity resistance as a single character could account for the limited success. We have already established that the salt tolerance of halophytes is a multi-genic trait and the same is true for non-halophytes (Flowers 2004). It is not our intention here to rehearse the arguments over approaches to breeding for complex traits. However, it is clear that breeding for yield has not delivered many salt-resistant varieties of the common crops (Flowers 2004; Witcombe *et al.* 2008). The probability of combining two complex traits – yield and salt tolerance – to maximise yield under saline conditions is evidentially very low. As a consequence, Yeo *et al.* (1990) advocated understanding the physiology of tolerance and pooling a range of traits an approach that had some success for rice (Dedolph and Hettel 1997). A similar approach has been advocated for durum wheat combining traits for regulating sodium accumulation and osmotic adjustment (Munns *et al.* 2002; James *et al.* 2006, 2008). The traits that necessarily need to be pooled for wheat (and barley) include Na 'exclusion', K/Na discrimination, the retention of ions in sheaths, tissue tolerance, ion partitioning into different leaves, osmotic adjustment, enhanced vigour, water use efficiency and early flowering (Colmer *et al.* 2005). In addition other traits such as tolerance to water-logging (see Colmer and Flowers 2008 for a discussion of flooding tolerance in halophytes) are vitally important, further complicating any breeding program (Colmer *et al.* 2005). Given the complexity of the task, it is not surprising that the transfer of one or two genes has generally failed to change the tolerance of transgenic plants; what is surprising is that there

are instances where such simple gene transfers appear to have altered yield (Flowers 2004).

Evolutionary studies of halophytes, which reconstruct the origin and development of salt tolerance in a variety of plant lineages, may help us to understand why artificial breeding has failed to produce robust and productive salt tolerant crops. Such studies may also help us develop new salt-tolerant lines by revealing the order of acquisition of components of salt tolerance, or indicating favourable genetic backgrounds on which salt tolerance may be developed. By examining the repeated evolution of this complex trait we may identify particular traits or conditions that predispose species to evolve a complex, multi-faceted trait such as salt tolerance and give rise to halophyte lineages. More generally, this may shed light on the adaptation of angiosperm lineages to extreme environments. In order to achieve these ends, more information is required on, at a minimum, the effects of salt on the growth and ion relations of a wider range of plant species that may prove to be halophytes.

Acknowledgements

HKG would like to thank the Egyptian Government for financial support while at the University of Sussex and our thanks also go to Jessica Thomas and Katherine Byron for assistance in earlier stages of this project.

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Manuscript received 6 November 2009, accepted 3 March 2010