

VIEWPOINT: PART OF A SPECIAL ISSUE ON HALOPHYTES AND SALINE ADAPTATIONS

Macroevolutionary patterns of salt tolerance in angiosperms

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• **Background** Halophytes are rare, with only 0.25 % of angiosperm species able to complete their life cycle in saline conditions. This could be interpreted as evidence that salt tolerance is difficult to evolve. However, consideration of the phylogenetic distribution of halophytes paints a different picture: salt tolerance has evolved independently in many different lineages, and halophytes are widely distributed across angiosperm families. In this Viewpoint, I will consider what phylogenetic analysis of halophytes can tell us about the macroevolution of salt tolerance.

• **Hypothesis** Phylogenetic analyses of salt tolerance have shown contrasting patterns in different families. In some families, such as chenopods, salt tolerance evolved early in the lineage and has been retained in many lineages. But in other families, including grasses, there have been a surprisingly large number of independent origins of salt tolerance, most of which are relatively recent and result in only one or a few salt-tolerant species. This pattern of many recent origins implies either a high transition rate (salt tolerance is gained and lost often) or a high extinction rate (salt-tolerant lineages do not tend to persist over macroevolutionary timescales). While salt tolerance can evolve in a wide range of genetic backgrounds, some lineages are more likely to produce halophytes than others. This may be due to enabling traits that act as stepping stones to developing salt tolerance. The ability to tolerate environmental salt may increase tolerance of other stresses or vice versa.

• **Conclusions** Phylogenetic analyses suggest that enabling traits and cross-tolerances may make some lineages more likely to adapt to increasing salinization, a finding that may prove useful in assessing the probable impact of rapid environmental change on vegetation communities, and in selecting taxa to develop for use in landscape rehabilitation and agriculture.

Key words: C₄ photosynthesis, comparative analysis, evolvability, grasses, halophyte, phylogeny, environmental stress tolerance, lability, salt tolerance, saline adaptations.

INTRODUCTION

Salt tolerance provides an interesting case study in the evolution of a complex trait that evolves in response to challenging environmental conditions. Salinity occurs in a wide range of environments, and presents challenges for plant growth, both directly, through toxicity of ions, and indirectly, by increasing osmotic stress. Yet a wide range of plant lineages have evolved traits that allow them to persist and complete their life cycles in saline conditions. Here, I ask what a macroevolutionary perspective can reveal about the evolution of salt tolerance, by examining the phylogenetic distribution of salt-tolerant species of flowering plants. This will be a broad view of salt tolerance and details of physiology, taxonomy and distribution will necessarily be lost. Furthermore, I will confine this discussion to inference from phylogenetic studies, without considering other macroevolutionary, macroecological or palaeobiological approaches. However, I hope that the loss of detail suffered in taking a broad-scale approach will be compensated for by revealing some larger-scale patterns that might not be obvious from more focused studies.

Four key observations inform this macroevolutionary view of salt tolerance in flowering plants. The first is that halophytes are rare – only 0.25 % of flowering plant species are known to

be able to complete their life cycle in saline conditions (Flowers *et al.*, 2010). This is despite the fact that naturally occurring saline habitats are not uncommon, with salt-affected soil comprising at least 2 % and as much as 10 % of the global land area (Rengasamy, 2006; Munns and Tester, 2008; Ruan *et al.*, 2010; Shabala, 2013). Salt-affected environments are widespread and varied, including coastal environments, salt marshes, salt lakes and many arid areas. Salt will tend to accumulate in the soil when evaporation is high relative to precipitation, such that salt will build up in the soil profile (Bui, 2013). The amount of salt-affected land is now increasing dramatically through land use practices that raise the water table and increase evaporation, such as irrigation and vegetation clearance (Rengasamy, 2006; Shabala, 2013).

The second key observation is that salt tolerance is taxonomically widespread. There are some clades that contain a large number of halophytes, such as the Chenopodiaceae (Kadereit *et al.*, 2012), and other clades that contain few or none, such as the Bambusiodeae (Bennett *et al.*, 2013). But rather than being confined to a few key specialist clades, halophytes occur throughout the flowering plants. At least one-third of all angiosperm orders contain one or more species known to be capable of completing its life cycle under saline conditions

(Flowers *et al.*, 2010). However, halophytes are in the minority in all orders, generally making up only a few per cent or less of the described species.

The third key observation is that salt tolerance appears to be a genetically complex trait. A large number of candidate genes have been identified, ranging from general stress response pathways to specific Na⁺ exclusion proteins (Ashraf and Foolad, 2012; Shavrukov, 2013). None of these genes in themselves confer salt tolerance; instead they modify existing physiological mechanisms to make plants more resistant to the stresses imposed by environmental salt. It seems reasonable to suppose that salinity tolerance is typically gained through changes to a large number of anatomical and physiological traits, such as production of compatible solutes to mitigate against osmotic stress, compartmentalization of toxic ions and excretion through specialized salt glands (Flowers and Flowers, 2005; Flowers and Colmer, 2008). Different species may use different mechanisms or combinations of traits, for example varying in their reliance on salt exclusion, salt accumulation or salt excretion (Munns and Tester, 2008). Life-history strategies can also play a role in salt tolerance, for example through dormancy during dry periods when soil salinity is highest, followed by emergence after rain when soil salinity drops (Cao *et al.*, 2014).

The fourth observation is that it has been remarkably difficult to breed or engineer increased salt tolerance into mainstream agricultural plant varieties. There have been notable exceptions, with improvements in salt tolerance of agricultural varieties through the introduction of single genes (Roy *et al.*, 2014), such as the *Nax1* and *Nax2* sodium transport genes in wheat (James *et al.*, 2012; Munns *et al.*, 2012). The genetic complexity of plant stress responses has been considered a barrier to development of crops that are robust to environmental stresses including salinity (Flowers and Flowers, 2005; Ruan *et al.*, 2010; Roy *et al.*, 2011; Ashraf and Foolad, 2012). In addition, investment in salt tolerance mechanisms may involve trade-offs, such as decreasing leaf area, that make salt-tolerant varieties commercially uncompetitive.

These observations present a paradox: saline habitats are common, so why are there relatively few halophytes? If salt tolerance is a complex trait that is difficult to breed into crop plants, then why has it evolved so many times in different plant groups? If salt tolerance evolves frequently in a wide range of lineages, then why are there relatively few large clades of halophytes? These are evolutionary questions, so in this review I will explore what we can gain by taking a macroevolutionary approach to understanding salt tolerance.

Many studies of salt tolerance focus on understanding the physiological or genetic mechanisms underlying salt tolerance in particular taxa, dissecting the effect of salt on growth or reproduction, or exploring the ecological consequences of environmental salt (e.g. Feldman *et al.*, 2008; Glenn *et al.*, 2012; Eallonardo *et al.*, 2013; Hamed *et al.*, 2013; Shabala, 2013). A macroevolutionary approach takes a broader view, where the focus is not on particular species or environments but on detecting any general patterns in the evolution of salt tolerance by comparing a large number of different lineages using phylogenetic comparative analysis. Clearly, this cannot replace detailed understanding of salt tolerance at the physiological level, or examination of the particular strategies employed by different species. But the hope is that macroevolutionary patterns could

reveal some of the underlying evolutionary forces that shape biodiversity and distribution of halophytes, which might then prompt more fine-scale studies that examine the links in more detail.

PHYLOGENETIC DISTRIBUTION OF SALT TOLERANCE

The distribution of halophytes on phylogenies can tell us much about the macroevolutionary dynamics of salt tolerance. In particular, we can estimate how often salt tolerance has evolved, whether it is more likely to evolve in some lineages than others and if the patterns of diversification of salt-tolerant lineages differ from their non-salt tolerant relatives.

Multiple origins of salt tolerance in grasses

To illustrate this approach, we analysed the patterns of occurrence of salt-tolerant species in the grass family (Poaceae). We focused on the grass family for several reasons: grasses are a large and diverse family with many recorded salt-tolerant species (Flowers *et al.*, 2006); there were several phylogenies that have a high degree of coverage, with one complete to genus level (Bouchenak-Khelladi *et al.*, 2010), and another 20 % complete to species level (Edwards and Smith, 2010); and grasses contain many of the economically important crops species that have been the focus of intense breeding efforts to increase salt tolerance, particularly wheat, rice and barley (e.g. Colmer *et al.*, 2006; Munns *et al.*, 2006; Ashraf and Foolad, 2012).

We assembled a list of reported salt-tolerant grass species and plotted them on a comprehensive phylogeny (Bennett *et al.*, 2013). We were surprised to find that, rather than clustering in groups of related species, salt-tolerant grass species were scattered throughout the phylogeny, such that most salt-tolerant species had relatively few identified salt-tolerant relatives. The most likely explanation for this pattern is that there have been many independent origins of salt tolerance in the grass family, each of which is relatively recent and has given rise to only one or a few salt-tolerant species. The 200 identified halophytic grasses have apparently arisen from over 70 different independent origins of salt tolerance. This ‘tippy’ pattern of many recent origins of salt tolerance, all at the tips of the phylogeny rather than deep in the tree defining large clades, is robust to changing the methods or assumptions of the analysis (Fig. 1). The tippy pattern was qualitatively the same whether we conducted the analysis on an incomplete species-level tree (Edwards and Smith, 2010) or a complete genus-level tree (Bouchenak-Khelladi *et al.*, 2010). The same pattern was found when considering a subset of halophytes known to have very high levels of salt tolerance, being able to complete their life cycle in conditions equivalent to >200 mM NaCl (approximately half the salinity of seawater: Flowers and Colmer, 2008).

Do other families contain many origins of salt tolerance?

Is this pattern of frequent gain of salt tolerance a peculiarity of grasses? Do other families show the extreme conservation of

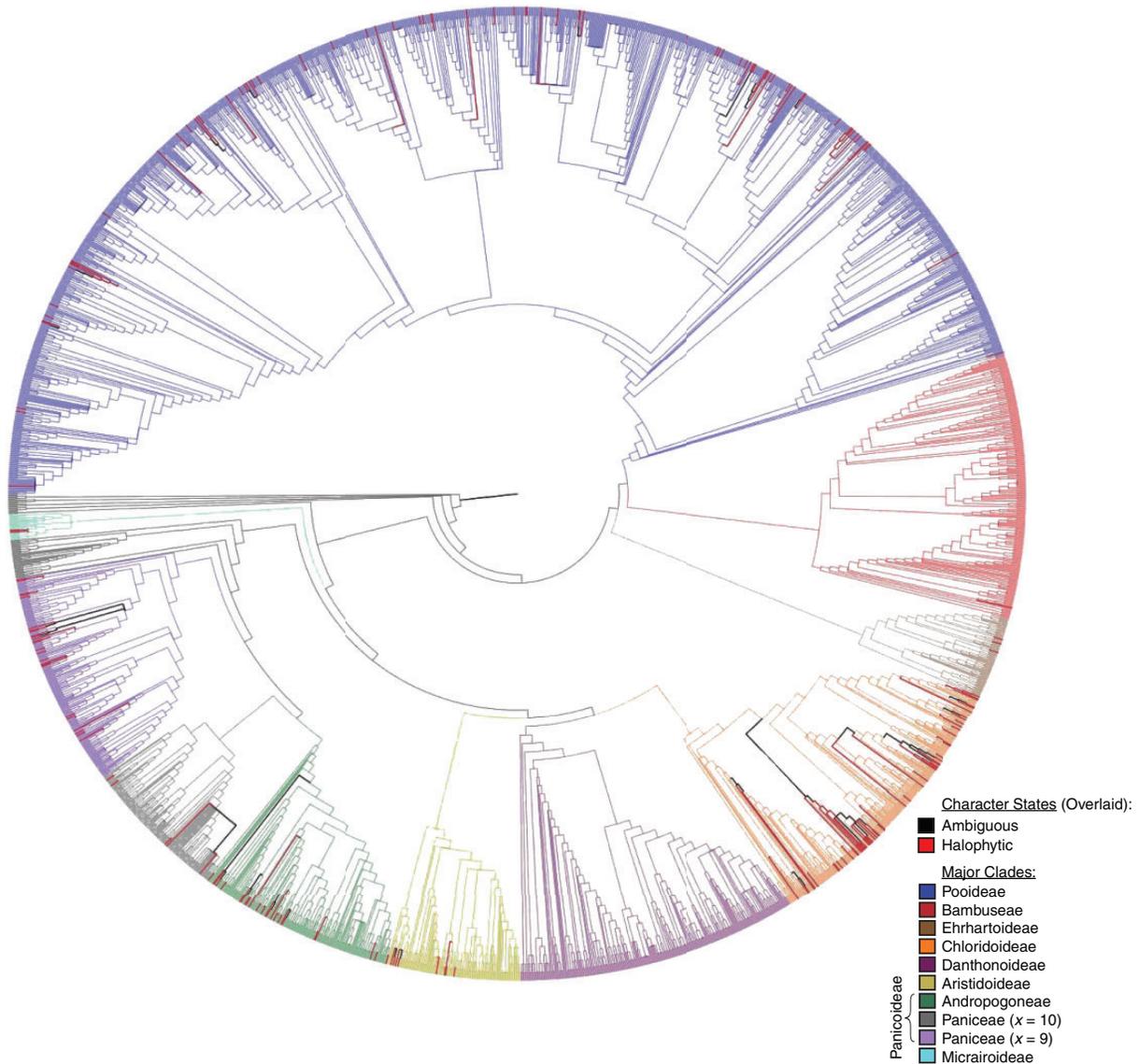


FIG. 1. Reconstructed origins of salt tolerance within the grass family (red branches) using unweighted parsimony. Black branches indicate those where it is equally parsimonious that the character state is either salt tolerant or non-tolerant. Most origins of salt tolerance are estimated to have led to a relatively small number of halophytes under unweighted parsimony. The phylogeny is based on [Edwards and Smith \(2010\)](#) but has been made ultrametric. Reproduced, with permission, from [Bennett \(2010\)](#).

salt tolerance seen in the chenopods? To see if either of these patterns is common in other angiosperm families, we selected 23 families of angiosperms for phylogenetic analysis. These were not selected to represent particular evolutionary patterns, but to provide family-level phylogenies of sufficient size and coverage, amenable to phylogenetic analysis of trait evolution. So our selection criteria were that test families needed to be able to be represented as a monophyletic clade of at least 25 taxa within a large published angiosperm phylogeny ([Smith *et al.*, 2011](#)), and each family tree needed to include at least eight recognized halophyte species.

We then reconstructed the inferred number of origins of salt tolerance, given its distribution on the phylogeny, using a parsimony criterion (this is a conservative approach as it infers the minimum number of origins needed to explain the observed

distribution). To investigate whether the distribution of salt tolerance is more ‘tippy’ than expected, we generated a null model of trait distribution by simulating the evolution of a trait along the phylogeny using a Brownian motion model ([Felsenstein, 2005](#); [Fritz & Purvis, 2010](#)). By repeating the simulation 1000 times for each family, we could derive an expected distribution of the number of origins given the observed taxa with the trait. In ten of the 23 sampled families, salt tolerance had a significantly higher number of evolutionary origins than expected given the number of halophytes in the family tree. Many of the other families tested showed a similar (non-significant) pattern of many independent origins each leading to only one or few extant halophyte species. One of the tested families (Tamaricaceae, salt cedars, in which most of the species are highly salt tolerant) showed significantly fewer origins of salt

tolerance than expected. This survey of patterns of salt tolerance across a range of angiosperm families suggests that pattern of frequent origins may be a common, but not universal, feature of the macroevolution of salt tolerance in flowering plants.

Possible macroevolutionary mechanisms

What can the tippy pattern of salt tolerance tell us about the tempo and mode of evolution of salt tolerance in these families? To investigate this further, we compared the phylogenetic pattern of halophytes in a subset of grasses, the core Pooideae, with null distributions generated under various evolutionary models, varying patterns of speciation and extinction rates, and the rates of gain and loss of salt tolerance. The best fitting model suggested that salt tolerance is labile with an enhanced rate of loss, but with a lower extinction rate. This suggests that salt tolerance in grasses is gained often in the pooid grasses, but is then typically lost again.

This analysis contributes to a picture that suggests that salt tolerance evolves often but fails to persist. So while salt tolerance seems to be surprisingly easy to achieve, it appears to be hard to maintain over macroevolutionary timescales (Edwards and Donoghue, 2013). The high loss rate of salt tolerance may be due to the costs incurred in building and maintaining defence against salt. Any investment in salt tolerance mechanisms, for example in production of compatible solutes or transport of excluded ions, will necessarily use resources that could have been put into growth (Yeo, 1983; Flowers, 1985; Cheeseman, 1988; Eallonardo *et al.*, 2013). As salinity increases, a point may be reached where reduction in growth, or increase in leaf death, becomes unviable or uncompetitive (Munns and Termaat, 1986; Denby and Gehring, 2005).

The phylogenetic patterns indicate that salt tolerance has been gained independently in a very wide variety of lineages, suggesting that many different kinds of plants have the capacity for evolving salt tolerance. But, although widely dispersed, salt tolerance is not randomly distributed across the angiosperm phylogeny (Flowers *et al.*, 2010; Bennett *et al.*, 2013). In other words, while many lineages contain halophytes, some contain more halophytes than we would expect if salt tolerance evolves with equal frequency in all lineages. There are several possible explanations for this significant phylogenetic clustering. One is that lineages differ in their exposure to saline environments, such that the non-random distribution of lineages in space leads to a concentration of halophytes in lineages found in areas more likely to contain environmental salt, such as arid areas (Edwards and Donoghue, 2013). Testing this idea would require a combination of biogeographical modelling and phylogenetic analysis.

An alternative explanation is that some lineages have particular background traits that make it easier for them to evolve salt tolerance. If we could identify these background traits, they might provide not only greater understanding into the evolution of salt tolerance but also potential targets for development of lineages for agriculture and phytoremediation. However, identifying these background traits is complicated by the tangled web of interconnected traits linking anatomical and physiological features with environmental distribution. Therefore, any test of the association between background traits and salt tolerance

must be conducted while allowing for patterns of co-variation generated by the process of descent with modification.

Controlling for phylogenetic covariation

Because they are inherited by many descendants from a common ancestor, we expect biological traits such as photosynthetic pathway and salt tolerance to tend to be more similar among close relatives. This non-independence of traits confounds attempts to compare species to determine whether two traits are causally linked, because relatives will tend to inherit both traits from their ancestors, whether or not the two traits are dependent on each other. To establish whether there is a meaningful evolutionary link between traits, beyond their incidental inheritance from a shared ancestor, we can compare multiple independent origins of each trait and ask whether the acquisition of one trait in a lineage makes it more likely to also acquire another trait.

For example, lineages with C₄ photosynthesis often contain many halophytes (Sage and Monson, 1999), and C₄ plants form a higher proportion of the vegetation in some saline habitats (e.g. Feldman *et al.*, 2008; Shuyskaya *et al.*, 2012; Eallonardo *et al.*, 2013). This pattern of association has been interpreted as a reflection of adaptation to water stress: by increasing water use efficiency, C₄ plants reduce the negative impacts of environmental salt on water stress and ion toxicity (Sage *et al.*, 2012). In this sense, C₄ photosynthesis could be considered an enabling trait for the evolution of salt tolerance. In turn, particular features of leaf anatomy can be considered as enabling traits for the repeated origins of C₄ photosynthesis. Like salt tolerance, C₄ photosynthesis has evolved many times (more than 60 origins in angiosperms), but more often in some lineages than others (Christin *et al.*, 2013). So lineages with particular features of leaf anatomy are more likely to evolve C₄ photosynthesis (Kadereit *et al.*, 2014), leading to a concomitant increased tolerance to water stress and salinity (Christin *et al.*, 2013). Conversely, lineages in arid environments, where they are more likely to encounter environmental salinity, are more likely to have C₄ photosynthesis (Kadereit and Freitag, 2011).

In grasses, halophytes are significantly more likely to occur in lineages with C₄ photosynthesis, and the pattern of salt tolerance and C₄ on the phylogeny suggests correlated evolution of salt tolerance and C₄ (Bromham and Bennett, 2014). C₃ lineages, by contrast, have significantly fewer halophytes than would be expected if salt tolerance was random with respect to photosynthetic pathway. This analysis demonstrates that C₄ photosynthesis and salt tolerance have a significant connection: their co-occurrence in species is neither explained by a random distribution nor explained simply as a matter of shared inheritance of unconnected traits. But this connection does not tell us whether C₄ increases the chance of salt tolerance, or salt tolerance increases the chance of C₄ evolving, or whether both traits are connected indirectly. The movement of grass lineages from shady forest environments to open, arid and salt-affected areas may have stimulated the evolution of more water-efficient C₄ photosynthesis, or the evolution of C₄ may have acted as a potentiating trait that allowed some grass lineages to move into these challenging habitats, or both (Feldman *et al.*, 2008; Osborne and Freckleton, 2009; Edwards and Smith, 2010; Liu *et al.*, 2012).

Challenges in inferring order of acquisition

It may seem obvious that in this ‘chicken and egg’ problem, C₄ must have come first, because there are fewer C₄ origins in the grass family, and they are deeper in the tree, defining large clades of related C₄ species. Most origins of salt tolerance, by contrast, are shallow in the tree and occur in C₄ lineages. But there are several limitations of using phylogenetic studies such as these to identify enabling traits for salt tolerance (Christin *et al.*, 2010).

The first is that differences in trait lability could create the impression of a directional relationship. Both C₄ photosynthesis and salt tolerance are labile on the grass phylogeny, but to different degrees: C₄ has been estimated to have arisen at least 22 times in grasses, but salt tolerance more than 70 times. The ‘tip-piness’ of salt tolerance on the grass tree is likely to be a reflection of this high transition rate. Most reconstructed origins of salt tolerance are relatively young, but this is unlikely to be because salt tolerance is only a recent invention. Instead, it implies that salt tolerance is constantly arising and being lost. We cannot directly reconstruct past origins of salt tolerance that have now been lost (through extinction or trait reversal) because they are not represented by any extant halophytic taxa. This problem is reflected in the difference between parsimony- and likelihood-based reconstructions of origins of salt tolerance. Because parsimony infers the minimum number of origins needed to explain the occurrence of salt tolerance in extant taxa, it reconstructs shallow origins near the tips of the tree, but a likelihood model will infer many more gains and losses throughout the internal branches of the phylogeny (Bennett, 2010). So although we can infer that halophytes evolve more often in C₄ lineages, we cannot rule out that C₄ photosynthesis evolved more often in lineages that were salt tolerant at the time (Fig. 2).

A contrasting pattern was observed for the Chenopodiaceae, which contains many salt-tolerant species. C₄ photosynthesis has evolved multiple times within this family, and, like the grasses, there is a significant association between salt tolerance and C₄ on the phylogeny. Because salt tolerance arose early in the history of this lineage, in this family salt tolerance precedes C₄ on the phylogeny (Kadereit *et al.*, 2012). This also reflects relative differences in trait lability: salt tolerance has been gained fewer times than C₄ in Chenopodiaceae, with few origins deep in the tree. Given that the phylogenetic pattern of C₄ in this family implies several reversals from C₄ to C₃, as for the grasses, we can infer that C₄ and salt tolerance are significantly associated, but we cannot prove whether salt tolerance increases the likelihood of gaining C₄, or vice versa, or whether the two are linked indirectly through another trait, for example adaptation to arid environments.

The second limitation is that salinity is distributed non-randomly in space, so the opportunity for a lineage to evolve salt tolerance is affected by where the lineage is located. As low water availability, high temperature and salinity tend to co-occur on the landscape (Bui, 2013), the correlation between C₄ photosynthesis and salinity could potentially be due to the likelihood of co-occurrence of salinity and other conditions that promote C₄ photosynthesis (Sage, 2004). As lineages are non-randomly distributed in space, some lineages may have more ‘evolutionary access’ to saline environments than others

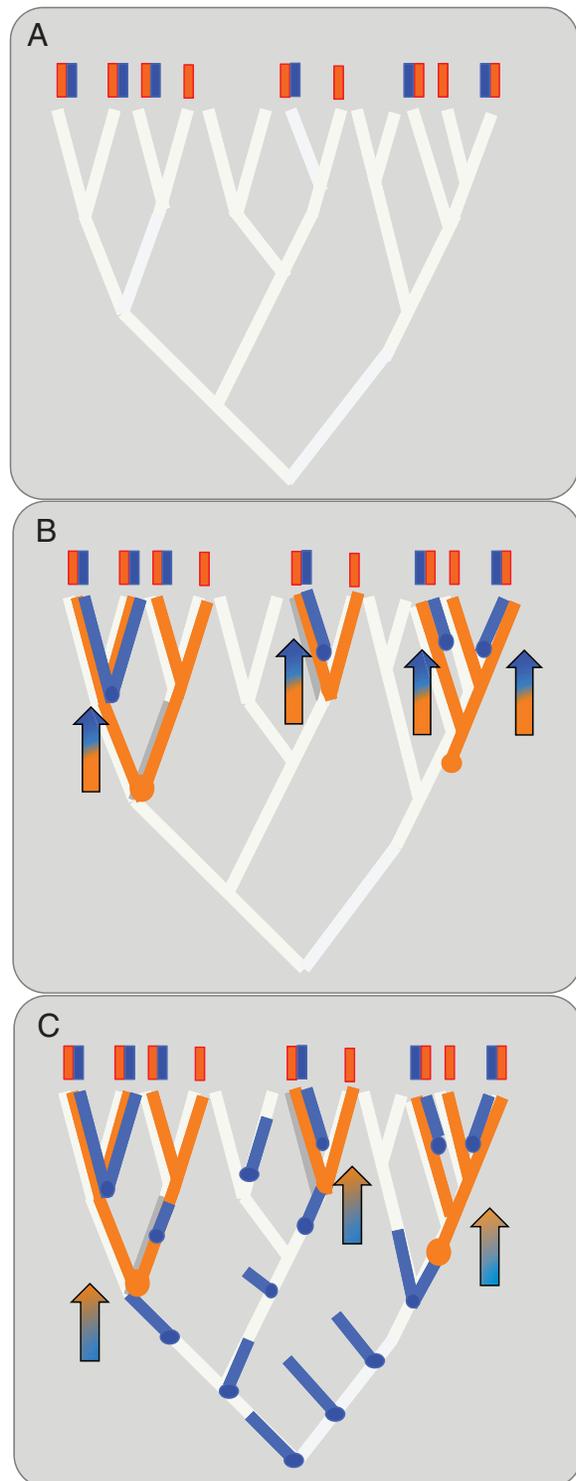


FIG. 2. Illustration of how trait lability affects reconstruction of trait order. In this hypothetical example, two traits (orange and blue) are found in a number of taxa on the tree (A). There are fewer, deeper origins of orange inferred, but more, recent origins of blue. One interpretation is that orange always precedes blue (B). But if blue has many recent origins, then one possible explanation is that it is relatively labile, being gained and lost often (by reversal or extinction), in which case it is possible that orange always arose in a blue lineage (C). In grasses, C₄ shows the orange pattern (fewer, deeper origins) and salt tolerance the blue pattern (more, shallower origins), but in chenopods the situation is reversed, with salt tolerance having fewer deeper origins than C₄.

(Edwards and Donoghue, 2013). For example, there are no known halophytic bamboos, but this may be, at least in part, because bamboos are generally forest specialists, so tend to be found in non-saline environments (Bennett *et al.*, 2013). Spatial accessibility seems unlikely to provide a general explanation for why some lineages develop many salt-tolerant species and some do not, but distribution may provide a confounding factor that needs to be considered when examining the macroevolution of salt tolerance. A spatially explicit test, accounting for phylogeny, is needed to tease out these interconnections.

STEPPING STONES TO SALT TOLERANCE

Given that salt tolerance is a complex trait that involves many different physiological and anatomical changes, how has it evolved so often in such a wide range of plant lineages? One potential explanation is that there are traits that provide the basis of stress tolerance, that may be common to many different adaptive strategies, and that plants with these traits will more rapidly adapt to environmental stress. This idea is referred to as the ‘stress tolerance syndrome’ (Chapin *et al.*, 1993). A similar but more general idea is that of an ‘enabling trait’ (Christin *et al.*, 2013) or ‘potentiating mutation’ (Lindsey *et al.*, 2013). Here, I will use the term ‘enabling trait’ to refer to any trait that does not in itself confer salt tolerance, but which makes it easier for a plant to evolve salt tolerance. Importantly, an enabling trait is neither necessary nor sufficient to develop salt tolerance, so we should expect to find many species with the enabling trait that are not salt tolerant, and many salt-tolerant traits without the enabling trait. But if we find a significant co-occurrence of the two traits, then we can begin to explore the possible causal links.

Some traits may provide a platform on which to build not only resistance to environmental salt but also a range of other environmental stresses. For example, several different environmental stresses, such as cold, drought and heavy metals, can trigger accumulation of leaf proline, and high leaf proline can increase resistance to salinity (Hare and Cress, 1997; Ashraf and Foolad, 2007; Hamed *et al.*, 2013). This suggests that evolution of frost or drought tolerance could incidentally increase the likelihood of tolerating environmental salt. Particular signalling pathways may trigger reactions that increase resistance to multiple stresses simultaneously; for example, a transcription factor that induces stomatal closure increases both salt and drought tolerance in rice (Huang *et al.*, 2009; Song and Matsuoka, 2009).

Identifying cross-tolerance mechanisms can have practical benefits in identifying species likely to be able to cope with harsh conditions. For example, halophytes can often tolerate higher levels of heavy metals than other plants, possibly because both metallophytes and halophytes must have mechanisms for dealing with toxic cation loads, for example by compartmentalization, excretion or activation of antioxidant systems (Hamed *et al.*, 2013; Rozema and Schat, 2013). Some of the same stress response pathways may be activated by both salt and heavy metals, leading to the possibility that genetic modifications may make cultivars more resistant to both stresses (e.g. Singh *et al.*, 2012). Cross-tolerance between salinity and heavy metals is a practical issue for phytoremediation

because degraded sites are likely to have multiple problems such as contamination with both salts and metals (Manousaki and Kalogerakis, 2011; Hamed *et al.*, 2013).

While cross-tolerance is typically studied at the level of individual physiology, for example in glasshouse experiments, phylogenetic studies may shed some light on patterns that might not be obvious from a more detailed, physiological approach. For example, if we find that halophytes are significantly more likely to occur in lineages with specific background traits, or in clades that contain species with other kinds of environmental tolerance, then we may be able to use phylogenies as a predictive tool (Saslis-Lagoudakis *et al.*, 2012). In this way, phylogenetic analysis may help to detect previously unrecognized halophytes, or identify lineages with an enhanced capacity to evolve salt tolerance. Phylogenetic analysis has been used to identify relatives of halophyte species as potential targets for further investigation (Joseph *et al.*, 2013), but given the lability of salt tolerance, it cannot be assumed that all close relatives will have similar tolerance (Liu *et al.*, 2012). However, phylogenetic analysis may identify lineages in which salt tolerance has frequently evolved, potentially because they have traits that mean that they do not have so far to go to achieve salt tolerance. So while the wide phylogenetic distribution of halophytes suggests that many plant lineages possess the foundation traits required for the evolution of salt tolerance, some may be much closer to salt tolerance than others, so require fewer or smaller steps to get there.

EVOLVING IN RESPONSE TO INCREASING SALINITY

The large number of recent origins of salt tolerance across the angiosperms raises the issue of the ‘evolvability’ of salt tolerance. Are some lineages more likely to be able to adapt to increasing salinization than others? Do some trait combinations allow a more rapid acquisition of salt tolerance than others? Exploring the evolvability of salt tolerance may have a practical relevance, given the rapid rate of salinization in many parts of the world. The effects of changing land use such as increasing irrigation, clearance of native vegetation and redistribution of surface and groundwater may be exacerbated by climate change, leading to salinization of both soil and water (Nielsen and Brock, 2009; Connor *et al.*, 2012). Given the speed and extent of these changes, it is pertinent to ask what factors govern the ability of a wide range of lineages to evolve to tolerate environmental salt.

Salt tolerance is a complex trait built upon adjustment of many aspects of physiology, anatomy and life history. Given the polygenic nature of salt tolerance, recent exploration of the rate of change in tolerance under rapidly changing environmental conditions might provide an interesting framework for considering plant species’ responses increasing salinization. ‘Evolutionary rescue’ occurs when step-wise adaptation allows a lineage to persist in conditions that would have been lethal to its recent ancestors (Bell, 2013). The likelihood of evolutionary rescue has been modelled by considering the chance that relevant mutations will arise in a finite (and often declining) population that will be sufficiently advantageous to allow rapid selective replacement (Gonzalez *et al.*, 2013). Evolutionary

rescue can also depend on variation already present in the population. For example, if experimental populations of bacteria are exposed to antibiotics, treatments that start at high dose will result in a much higher rate of population extinction than those that build slowly over many generations to the same dose (Lindsey *et al.*, 2013). Gradual increase in antibiotic exposure allows the opportunity for mutations that confer partial resistance to arise and become fixed in the population. These potentiating changes do not in themselves confer resistance to the high doses, but they provide a platform on which to build high resistance. Similarly, yeasts are more likely to adapt to high salt if previously exposed to lower salt concentrations (Gonzalez and Bell, 2013). Speed of change is critical: if the environment changes too rapidly there may be insufficient time for potentiating mutations to build a foundation for resistance in the population. A focus on evolutionary rescue has led to hope that it may be possible to predict which lineages can persist in the face of rapid climate change and which are unlikely to have the capacity for change (Gonzalez *et al.*, 2013). For example, evolution of tolerance to heavy metals can occur rapidly, as demonstrated by the emergence of plants that can grow on mine tailings. But the diversity of plants growing on tailings is much lower than the surrounding area: only some lineages seem able to adapt to this rapid change to very challenging conditions (Bradshaw, 1991; Bell, 2013).

In evaluating which lineages have the capacity to evolve to cope with previously intolerable levels of salinity, the identification of enabling traits that provide a foundation for the development of salt tolerance could prove useful. In particular, order of acquisition of traits underlying salt tolerance could provide valuable information for targeted breeding, as it may be possible to build sequentially on key traits to increase productivity under saline conditions (Rozema and Schat, 2013). For this to work, we would need more detailed breakdown of the component traits of salt tolerance. In the studies reported here, we have taken a broad-brush approach, considering only whether a species can persist in saline conditions or not. But if sufficiently detailed information was available for many salt-tolerant species and (importantly) their non-salt-tolerant relatives, we could use phylogenetic analysis to explore the evolutionary pathways to increased salt tolerance. This in turn might allow a predictive model where we could identify non-salt-tolerant lineages that have many of the necessary components for increasing tolerance of environmental salt. Macroevolutionary perspectives may also be able to add a longer term view to this picture, by considering the balance between trait lability (how often the trait arises) and population persistence (whether lineages with that trait are able to persist and diversify in the long term: Edwards and Donoghue, 2013).

Agricultural studies of salt tolerance have naturally focused on discovering genes that each have a large impact, in the hope that salt tolerance can be achieved through targeted genetic manipulation, either through transgenesis of target genes into crop varieties or by marker-assisted breeding targeting genetic variation in crop plants and their relatives (e.g. Yamaguchi and Blumwald, 2005; Tester and Langridge, 2010). The picture emerging from macroevolutionary studies of halophytes is that salt tolerance arises frequently, potentially building on a background of traits that are also genetically complex. While these layers of complexity may appear to be bad news for genetic

manipulation, the good news is that there may be a large number of lineages that, while not halophytes, already have most of the bases for developing higher salt tolerance. Identifying suites of enabling traits might also lead to an understanding of the order of acquisition of traits. For example, it has been suggested that most halophytes have evolved from arid-adapted species (Rozema and Schat, 2013): if this is the case, then xerophytes might provide as good a foundation for developing salt-tolerant lineages as recognized halophytes. Understanding the interactions between different stress tolerances may be critical in evaluating whether future changes will push species into intolerable combinations of conditions (e.g. combined waterlogging and salinity: Bennett *et al.*, 2009).

CONCLUSIONS

Salt tolerance has evolved many times in a wide range of different lineages, a pattern that suggests that many lineages have the capacity for evolving increased salt tolerance. However, in many angiosperm families, salt-tolerant lineages do not appear to persist and diversify through macroevolutionary time, suggesting that salt tolerance may come at a cost that makes it unsustainable in the long term. While all plants may have some capacity to evolve greater salt tolerance, it may be easier to produce salt-tolerant lineages from some starting points than others. Traits that act as stepping stones to salt tolerance might make the path to increased salt tolerance a little easier to traverse. Increasing demand for food and fresh water will both contribute to and be limited by increasing salinization. Two strategies employed thus far have to been to try to develop naturally salt-tolerant species for agriculture or landscape remediation, or to increase the salt tolerance of glycophytic crops. Macroevolutionary patterns might help inform a third way, which is to identify lineages that are neither halophytes nor crop plants, but that are close to being both.

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