JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12320

SHORT COMMUNICATION

Salt tolerance evolves more frequently in C_4 grass lineages

L. BROMHAM & T. H. BENNETT

Division of Ecology, Evolution and Genetics, Centre for Macroevolution and Macroecology, Research School of Biology, Australian National University, Canberra, ACT, Australia

Keywords:

comparative;
evolution;
halophyte;
PACMAD;
phylogeny;
Poaceae;
salinity;
trait lability.

Abstract

Salt tolerance has evolved many times in the grass family, and yet few cereal crops are salt tolerant. Why has it been so difficult to develop crops tolerant of saline soils when salt tolerance has evolved so frequently in nature? One possible explanation is that some grass lineages have traits that predispose them to developing salt tolerance and that without these background traits, salt tolerance is harder to achieve. One candidate background trait is photosynthetic pathway, which has also been remarkably labile in grasses. At least 22 independent origins of the C_4 photosynthetic pathway have been suggested to occur within the grass family. It is possible that the evolution of C_4 photosynthesis aids exploitation of saline environments, because it reduces transpiration, increases water-use efficiency and limits the uptake of toxic ions. But the observed link between the evolution of C_4 photosynthesis and salt tolerance could simply be due to biases in phylogenetic distribution of halophytes or C_4 species. Here, we use a phylogenetic analysis to investigate the association between photosynthetic pathway and salt tolerance in the grass family Poaceae. We find that salt tolerance is significantly more likely to occur in lineages with C_4 photosynthesis than in C_3 lineages. We discuss the possible links between C_4 photosynthesis and salt tolerance and consider the limitations of inferring the direction of causality of this relationship.

Introduction

The amount of salt-affected land, currently over 6% of the land surface area, is increasing through agricultural practices and land clearance. Irrigated land, which produces a third of the world's food, is particularly prone to salinization: between 20 and 50% of the world's irrigation schemes are salt-affected (Flowers, 2004; Flowers *et al.*, 2010; Munns, 2011). Understanding the evolution and maintenance of salt tolerance in plants may help to develop strategies for utilizing and managing salt-affected land.

There are over 350 naturally halophytic (salt tolerant) grass species and subspecies. These halophytic grasses are not clustered in clades of related species, all descended from a few independent origins of salt tolerance. Instead, salt tolerance has evolved frequently in a large number of different lineages. A recent study estimated that there have been over 70 independent origins of salt tolerance in the grass family (Bennett *et al.*, 2013). Yet, although there is considerable benefit to producing crop plants that can grow on salt-affected land (Glenn *et al.*, 1999; Rozema & Flowers, 2008), there have been few commercially viable salt-tolerant cereal crops produced (Flowers & Yeo, 1995; Flowers & Flowers, 2005).

Why has it been so difficult to breed salt tolerance into cereal crops when it has evolved so many times within the grass family? There are several possible explanations (which are not mutually exclusive). Firstly, it may be that salt tolerance is a physiologically costly trait so that it is difficult to develop a productive crop plant that can produce commercially viable yields while dealing with environmental salt. Secondly, salt tolerance is a genetically complex trait, which may not present an easy target for breeding programs or genetic

Correspondence: L. Bromham, Division of Ecology, Evolution and Genetics, Centre for Macroevolution and Macroecology, Research School of Biology, Australian National University, Canberra ACT 0200, Australia. Tel.: +61 2 61259545; fax: 61 2 61255573; e-mail: Lindell.Bromham@anu.edu.au

manipulation (Roy *et al.*, 2011). Thirdly, salt tolerance may be more easily acquired with particular backgrounds as starting points, and thus will evolve more easily in certain lineages that already have these traits. It is this third possibility that we wish to examine in this study.

One possible background trait that may enhance the capacity to evolve salt tolerance is photosynthetic pathway. The C_4 mechanism of carbon fixation is a modified version of the ancestral (C_3) photosynthetic pathway, and it has evolved independently over 60 times in angiosperms (Sage *et al.*, 2012), including an estimated 22–24 gains within the grass family (Edwards & Smith, 2010; Grass Phylogeny Working Group II, 2012). By increasing the efficiency of carbon fixation, C_4 plants can reduce photorespiration and thus allow higher water-use efficiency and productivity. Therefore, C_4 photosynthesis has been assumed to have advantages under conditions that promote photorespiration, such as heat, drought, salinity and low atmospheric CO_2 (Sage & Monson, 1999; Sage, 2004; Sage *et al.*, 2012; Christin *et al.*, 2013).

Plants with C_4 photosynthesis are often found in salt-affected areas, and taxa with C_4 carbon fixation appear to be overrepresented among halophytes (Aronson, 1989; Sage & Monson, 1999; Dajic, 2006; Eallonardo *et al.*, 2013). However, the association between photosynthetic pathway and salt tolerance needs to be formally tested within a phylogenetic framework in order to account for confounding factors (Christin *et al.*, 2009; Osborne & Freckleton, 2009; Taylor *et al.*, 2010). C_4 species are nonrandomly distributed in the grass phylogeny (Table 1), with all known C_4 species occurring in the large ‘PACMAD’ clade, which contains the subfamilies Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae (Christin *et al.*, 2009, 2013). So even if a disproportionate number of halophytic grasses use C_4 photosynthesis, it is unclear whether this is due to a specific association between the two traits, or because there is some other feature of the PACMAD clade that increases the likelihood of evolving salt tolerance (Edwards *et al.*, 2007). Here, we ask whether C_4 photosynthesis is specifically associated with the evolution of salt tolerance, in order to shed light on some of the factors that have allowed some grass lineages to adapt to saline environments.

Table 1 The halophytes include in this study, as a proportion of the number of species represented in the phylogeny of Edwards and Smith (2010). There are proportionally more halophytes in the PACMAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae, Danthonioideae), which contains both C_3 and C_4 taxa, than there are in the BEP clade (Bambusoideae, Ehrhartoideae, Pooideae), which contains only C_3 lineages.

Clade		Species	Halophytes	Proportion
BEP	C_3 only	1526	87	0.057
PACMAD	C_3 and C_4	1143	118	0.103

Materials and methods

A list of halophytic grasses was taken from Bennett *et al.* (2013). Most studies use a standard definition of a halophyte as any species that can successfully complete its life cycle in saline conditions similar to those encountered in the natural environment, where saline conditions are defined as those where the soil solution has an electrical conductivity equivalent to ~80 mM NaCl at saturation, following Aronson (1989). However, this precise definition can rarely be applied in practice as the exact level of tolerance is typically known only for species that have been closely studied in the laboratory (Flowers, 2004). In most cases, it is necessary to rely on reports of populations growing in saline conditions in the field (see Bennett *et al.*, 2013). For example, the eHALOPH database (http://www.sussex.ac.uk/affiliates/halophytes) lists some species with specific ranges of soil electrical conductivity, but other species according to discrete categories such as xerohalophyte (e.g. inland salt desert species) or hydrohalophyte (e.g. tidal swamp or salt marsh species).

We used the molecular phylogeny published by Edwards and Smith (2010), which includes 2684 taxa (approximately 20% of all grass species). Two hundred of these taxa were identified as halophytes, following Bennett *et al.* (2013). To test the generality of patterns, all analyses were run both on the phylogeny of all Poaceae and also on a subtree containing the PACMAD clade only. Information on the photosynthetic pathways of all grasses in the phylogeny was also taken from Edwards and Smith (2010). To test that our results are not the result of sampling bias in Edwards and Smith data set, we also combined a list of all grass genera containing halophytes (see Bennett *et al.*, 2013; Table S2) with the complete genus-level phylogeny and photosynthetic pathway data set of Bouchenak-Khelladi *et al.* (2010).

A randomization test was conducted to test whether more halophytes occur in C_4 clades than expected by chance. A null distribution of the expected number of halophytes occurring in C_4 lineages was generated by randomly reassigning character states (200 halophytic/2484 nonhalophytic) across the tips of the phylogeny, then counting the number of these that fell on C_4 taxa. The randomization was repeated 10 000 times. We then compared the observed number of halophytes in C_3 and C_4 clades to this null distribution. The association between photosynthetic pathway and salt tolerance was deemed to be significantly different from chance when the observed number of halophytes was greater or less than in 95% of randomizations.

The correlation between salt tolerance and photosynthetic pathway was tested using Pagel's (1994) correlation analysis for discrete characters, as modified by Maddison and Maddison (2006). We analysed the transition rate between two states for the two characters that is between salt tolerant and salt sensitive, and between C_3 and C_4. This method estimates the fit of a Markov model where the rate of change in each character is independent of the state of the other and compares it to the fit of a correlated, state-dependent model, where the rate of transition from salt sensitive to salt tolerant is dependent on the type of photosynthetic pathway. If the state-dependant model fits significantly better, then this suggests that the evolution of the two traits is correlated. The significance of the likelihood difference between the models is estimated by comparison with simulated data. We used the 'Pagel94 correlation analysis' function of mesquite, optimizing likelihoods with ten iterations and estimating significance from 1000 simulations (Maddison & Maddison, 2006) using a maximum likelihood omnibus test as described by Pagel (1994). The optimal scaling of the *kappa* parameter was selected with the best fit to equal branch lengths (see Bennett *et al.*, 2013). The rate of evolution of salt tolerance in C_3 and C_4 clades was compared using the estimated rates of these transitions.

Results

The phylogenetic distribution of salt tolerance and C_4 photosynthesis is shown in Fig. 1.

Significantly more halophytes occur in C_4 lineages than if salt tolerance was random with respect to photosynthetic pathway, both across the whole Poaceae, and within the PACMAD clade (Table 2). For example, there are only three identified C_3 halophytes in the PACMAD clade of the species-level phylogeny, where we would expect 20–60 halophytes to occur in the C_3 lineages of the PACMAD if salt tolerance was randomly distributed on the phylogeny (Fig. 2).

Because not all grass species are included in our analysis, we checked that our results were not an artefact of undersampling halophytes in C_3 clades. Using the list of all grass genera containing halophytes

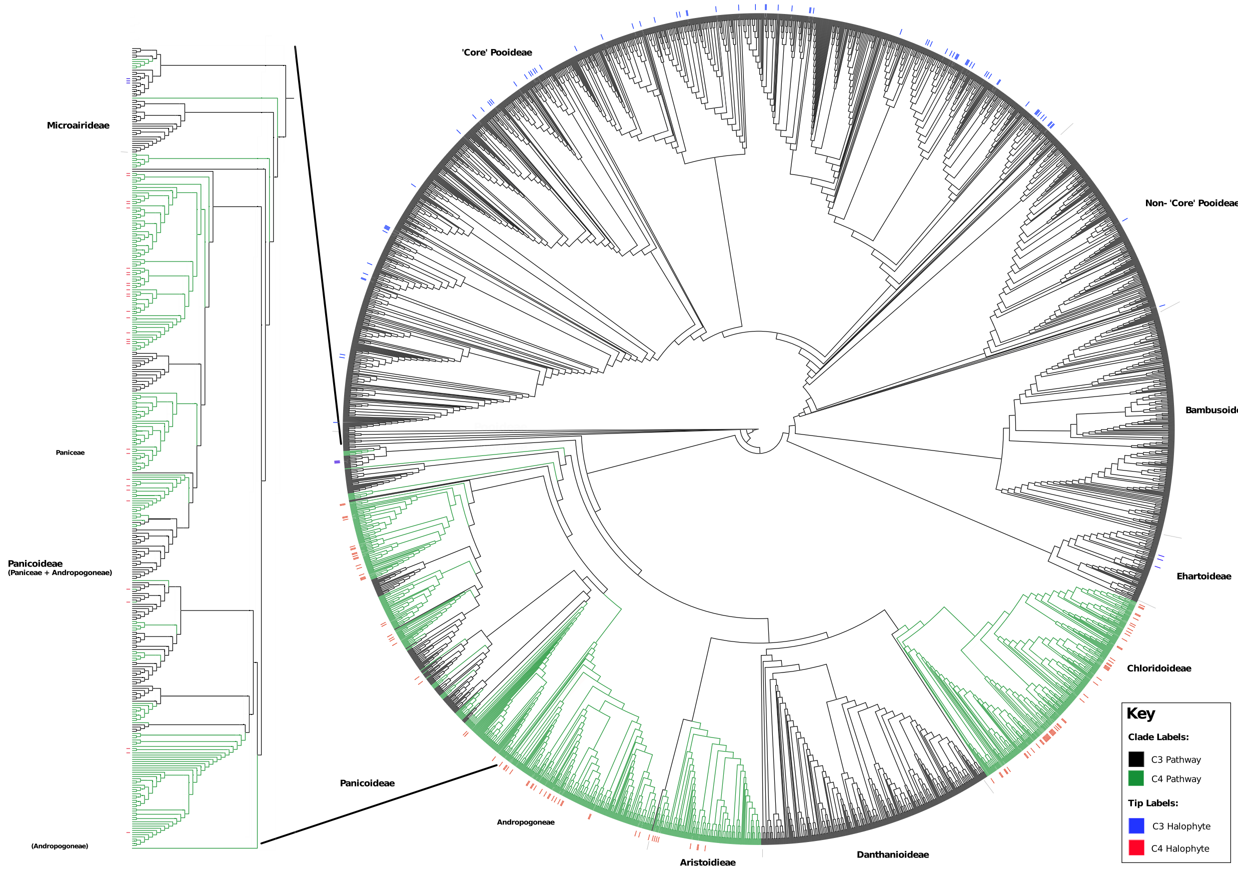

Fig. 1 Distribution of halophytes (salt-tolerant species) on the grass family, mapped onto the evolutionary pattern of C_4 photosynthesis estimated by Edwards and Smith (2010).

Table 2 Results of the correlation analyses conducted on both the whole grass family (Poaceae) and on the PACMAD clade, comparing the fit of a model where the evolution of C_4 and salt tolerance is correlated with a model to one where they are uncorrelated. The estimates are log likelihood (−lnL) of the correlated and uncorrelated models; difference in likelihoods (ΔlnL) of the two models; *P* values derived from 1000 simulations; estimated rate of gain of salt tolerance in C_3 and C_4 clades.

Clade	−lnL (uncorrelated)	−lnL (correlated)	ΔlnL	*P*	C_3 Rate	C_4 Rate
Poaceae	822.2	798.5	23.7	< 0.001	0.023	0.074
PACMAD	466.0	438.0	28.0	< 0.001	0.010	0.075

(Bennett *et al.*, 2013) and the complete genus-level phylogeny and photosynthetic pathway data set of Bouchenak-Khelladi *et al.* (2010), we found only one C_3 halophytic species from the PACMAD that was not included in our species-level analysis (*Rytidosperma rufum*), but many more C_4 halophytes not in the species-level phylogeny. This suggests that undersampling of C_3 halophytes has not biased this analysis.

If the overrepresentation of halophytes in the PACMAD clade was due to some feature of PACMAD other than photosynthetic pathway, then we would expect to observe many salt-tolerant C_3 species in the PACMAD. However, there are significantly fewer C_3 halophytes in the PACMAD (observed = 3) than expected on the basis of chance (expected > 20: Fig. 2). Instead, almost all C_3 halophytes on the phylogeny are found in the BEP clade (Bambusoideae, Ehrhartoideae, Pooideae), particularly in the core Pooideae. Therefore, we can conclude that PACMAD lineages with C_4 photosynthesis are more likely to contain salt-tolerant species than C_3 lineages in the PACMAD, a pattern confirmed by the correlation analyses.

The correlation analyses indicated a significant association between salt tolerance and C_4 photosynthesis on the species-level phylogeny of grasses (Table 2). The correlated model, which allows the rate of transition from salt sensitive to salt tolerant to vary according to the state of the photosynthetic pathway, fits the data significantly better than the uncorrelated model, for both the whole Poaceae family ($\Delta\ln L = 23.7$, $P < 0.001$) and for the PACMAD clade ($\Delta\ln L = 28.0$, $P < 0.001$). For the phylogeny of all Poaceae, the best-fitting model gave an estimate of the rate of gain of salt tolerance in C_4 clades that was approximately three times higher than the estimated rate of gain of salt tolerance in C_3 clades. The analysis on only the PACMAD clade gave even more pronounced results, with an estimated rate of gain of salt tolerance in C_4 clades approximately seven times higher than the estimated rate of gain of salt tolerance in C_3 clades (Table 2).

Discussion

Although it has often been proposed that plants with C_4 photosynthesis are more likely to be able to adapt to live in saline habitats, this hypothesis has not been robustly tested before. Using a broad-scale comparative approach, we show that halophytic grasses are significantly more likely to occur in lineages with C_4 photosynthesis than expected if salt tolerance was random with respect to photosynthetic pathway. Salt tolerance appears to have evolved repeatedly within many C_4 grass clades, with salt tolerance arising at a more frequently in C_4 lineages than in C_3 groups. In fact, salt tolerance has evolved relatively rarely in C_3 lineages outside of the 'core' Pooideae (Edwards & Smith, 2010). But the observation of this significant correlation does not, by itself, establish a direct causal connection between the two traits, nor the direction of causality. Does salt tolerance favour the evolution of C_4 photosynthesis, does C_4 promote the evolution of salt tolerance, or are both traits linked indirectly, for example, by tending to co-occur in taxa adapted to open and arid habitats?

Taken at face value, our results could be interpreted as evidence that, within the grass family, C_4 lineages have been more likely to develop salt tolerance than C_3 lineages. There are far more origins of salt tolerance within the grasses (around 70) than origins of C_4 (around 20), and the gains of salt tolerance tend to be distributed near the tips of the phylogeny and are therefore relatively recent (Bennett *et al.*, 2013). This pattern is compatible with the hypothesis that the adoption of C_4 photosynthesis allowed expansion into arid and saline habitats (Stromberg, 2011); therefore, C_4 lineages were more likely to produce halophytic species (Osborne & Freckleton, 2009). C_4 lineages may be an advantageous starting point for the evolution of salt tolerance, given that the greater water-use efficiency of C_4 photosynthesis lowers the flux of water and salts through the plant per growth unit, which can reduce the amount of salt that a plant has to exclude, compartmentalize, or secrete for a given amount of carbon fixation (Sage, 2001).

An alternative explanation of this link between photosynthetic pathway and salt tolerance is that lineages adapted to saline environments may be more likely to evolve C_4 photosynthesis. It has been argued that the adaptation to harsh environments, such as arid or saline habitats, has promoted selection for C_4 photosynthesis, by favouring traits that reduce ionic stress through decreasing transpiration rates. For example, Kadereit *et al.* (2012) estimated that there have been 10 origins of C_4 photosynthesis in the Chenopodiaceae,

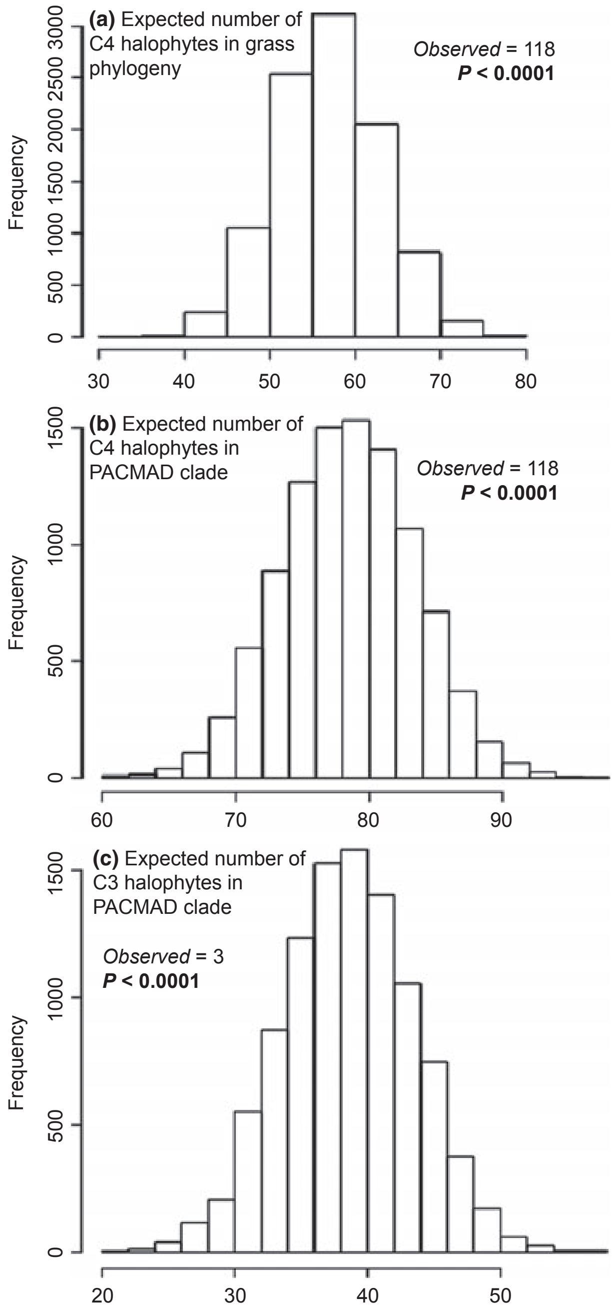

Fig. 2 Distribution of expected numbers of halophytes in each clade if salt tolerance occurred independently of photosynthetic pathway. In each case, the observed value is not contained in the distribution of expected values, so the null model of chance association between salt tolerance and photosynthetic pathway can be rejected. The test was repeated on both the whole grass phylogeny and on the subclade containing the PACMAD families (see Materials and methods for details). Because all C_4 halophytes occur within the PACMAD clades, the observed number of C_4 halophytes is the same for both the whole family and the PACMAD.

a clade of the Amaranthaceae containing many halophytes, but probably only one ancestral origin of salt tolerance. However, as with this study, inference of direction of causality may be conflated with differences in lability between traits.

Although we can be confident of a significant evolutionary link between salt tolerance and C_4 photosynthesis, the direction of the relationship cannot be easily inferred from the phylogenetic pattern alone. This is because different patterns of trait lability could create the false impression of a directional causal relationship. If salt tolerance is relatively labile in grasses (see Bennett *et al.*, 2013), then although most extant halophytic lineages have relatively recent origins, we can expect that salt tolerance has been gained and lost throughout the history of the grasses. Because we cannot directly reconstruct past evolution and loss of salt tolerance, we cannot rule out that C_4 photosynthesis has typically arisen in lineages growing under saline conditions, then some of those C_4 lineages lose salt tolerance or move to different habitats. So the order of acquisition may be an artefact of trait lability: C_4 photosynthesis may appear deeper in the tree, and therefore to have been gained first, because it evolves less often and is lost less often than salt tolerance.

Regardless of the direction of causality of the link between photosynthetic pathway and salt tolerance, these two hypotheses – C_4 promotes evolution of salt tolerance vs salt tolerance promoting evolution of C_4 – are not mutually exclusive. Ongoing adaptation to allow exploitation of open, arid and saline habitats may have resulted in the promotion of both salt tolerance and C_4 photosynthesis. Although responses to salinity are distinct from responses to aridity, mechanisms of tolerance to these stresses have much in common (Munns, 2002; Des Marais & Juenger, 2010), so it is possible that adaptation to aridity provides enabling conditions that promote salinity tolerance (or vice versa).

It is also possible that an indirect link between C_4 and salt tolerance could arise through ecological preference or biogeographic patterns. C_4 grasses in the PACMAD are more frequently found in open and arid habitats than C_3 PACMAD species (Osborne & Freckleton, 2009; Pau *et al.*, 2012). As highly saline soils do not generally support closed-canopy vegetation (mangrove forests being a notable exception), halophytes will also tend to occur in open habitats. Saline soils are also particularly prevalent in arid and semi-arid regions, so the large number of halophytes in C_4 clades may be explained by their inhabiting the general areas where salinity is more prevalent. Edwards and Donoghue (2013) point out that although the biased frequencies of transitions to C_4 across the grasses may be due to anatomical enablers, because the large bundle sheath cells of PACMAD grasses could give them a natural advantage over in evolving C_4 photosynthesis, it may be also the case that Pooid grasses tend to be distributed in cooler climates, and there is less advantage to evolving C_4 photosynthesis than there is for the PACMAD lineages (Edwards & Still, 2008; Edwards & Donoghue, 2013).

However, adaptation to open, arid conditions does not itself appear to be sufficient for a group to develop salt tolerance. For example, the subfamily Danthonioideae contains nearly 300 species of tussock and pampas grasses, found mainly in the Southern Hemisphere (Linder *et al.*, 2010). Many species in this subfamily are found in open and relatively arid habitats (Bouchenak-Khelladi *et al.*, 2010; Edwards & Smith, 2010), but the subfamily shows a paucity of halophytes. Across the angiosperms, there are other examples of arid-adapted groups that have rarely evolved salt tolerance, such as the Proteaceae and Cactaceae (Flowers *et al.*, 2010). Conversely, not all halophytes occur in open, arid environments, for example plants adapted to coastal salt marshes and mangrove forests. However, the potential for reduced transpiration rates in C_4 plants may be an advantage in salt-affected habitats even where water is not limited, as it may limit the physiological stress of osmotic adjustment.

It is interesting to note that some C_4 plants require small amounts of Na^+ for growth (Brownell & Crossland, 1972), and so do not thrive in the absence of Na^+ (Subbarao *et al.*, 2003). Some C_4 plants can use sodium ions as osmoticum to allow rapid grown under saline conditions (Kronzucker *et al.*, 2013). Sodium ions can also play a role in the concentration of CO_2 in C_4 physiology through Na^+-coupled pyruvate transport in chloroplasts (Furumoto *et al.*, 2011). However, the requirement for Na^+ for growth is not universal in C_4 plants, and some C_4 grasses, including maize and sugarcane, show no growth benefits from presence of sodium (Subbarao *et al.*, 2003).

Further studies are required to tease apart the intercorrelation of aridity, salinity, C_4 photosynthesis and salt tolerance. It would also be interesting to see whether C_4 photosynthesis is more commonly associated with the evolution of particular strategies of salt tolerance. For example, salt-tolerant grasses may rely more heavily on salt exclusion than dicotyledonous halophytes (Glenn *et al.*, 1999), and it could be that C_4 photosynthesis is particularly beneficial to this strategy of tolerance and less beneficial to other strategies such as salt accumulation. This could be tested by finding the correlation between specific salt tolerance traits and the C_4 pathway, both within the grass family, as well as in other families.

The significant correlation between C_4 and salt tolerance in naturally occurring grass species suggests that C_4 photosynthesis may provide advantages to the development of plant varieties that can grow in salt-affected areas. It is interesting to contrast the evolutionary and agricultural development of salt tolerance and C_4 photosynthesis. There is a growing effort to engineer the C_4 pathway into C_3 crop species to increase their yields (Sage & Zhu, 2011; von Caemmerer *et al.*, 2012). Hibberd *et al.* (2008) stated that ‘although generating C_4 rice is extremely ambitious, the polyphyletic evolution of C_4 photosynthesis provides cause for optimism’. The evolutionary lability of both C_4 photosynthesis and salt tolerance in grasses, and the apparent link between the two, might be considered encouraging, even if engineering C_4 photosynthesis or salt tolerance in commercially viable crops may be more difficult than some have hoped (e.g. Flowers, 2004; Flowers & Flowers, 2005; Zhu *et al.*, 2010).

Acknowledgments

We are grateful to Colin Osborne and co-authors for providing us with data on photosynthetic pathways, Erika Edwards and co-authors for providing the phylogeny, and to Rana Munns, Tim Flowers, Haris Saslis-Lagoudakis, Camile Moray, Marilyn Ball, Susanne von Caemmerer and Bas Bruning for helpful feedback on the manuscript.

References

Aronson, J.A. 1989. *HALOPH: A Data Base of Salt Tolerant Plants of the World*. Office of Arid Land Studies, University of Arizona, Tucson, Arizona.

Bennett, T.H., Flowers, T.J. & Bromham, L. 2013. Repeated evolution of salt-tolerance in grasses. *Biol. Lett.* **9**: 20130029.

Bouchenak-Khelladi, Y., Verboom, G.A., Savolainen, V. & Hodkinson, T.R. 2010. Biogeography of the grasses (Poaceae): a phylogenetic approach to reveal evolutionary history in geographical space and geological time. *Biol. J. Linn. Soc.* **162**: 543–557.

Brownell, P. & Crossland, C. 1972. The requirement for sodium as a micronutrient by species having the C4 dicarboxylic photosynthetic pathway. *Plant Physiol.* **49**: 794–797.

von Caemmerer, S., Quick, W.P. & Furbank, R.T. 2012. The development of C4 rice: current progress and future challenges. *Science* **336**: 1671–1672.

Christin, P.A., Salamin, N., Kellogg, E.A., Vicentini, A. & Besnard, G. 2009. Integrating phylogeny into studies of C4 variation in the Grasses. *Plant Physiol.* **149**: 82.

Christin, P.-A., Osborne, C.P., Chatelet, D.S., Columbus, J.T., Besnard, G., Hodkinson, T.R. *et al.* 2013. Anatomical enablers and the evolution of C4 photosynthesis in grasses. *Proc. Natl. Acad. Sci.* **110**: 1381–1386.

Dajic, Z. 2006. Salt stress. In: *Physiology and Molecular Biology of Stress Tolerance in Plants* (K.V. Madhava Rao, A.S. Raghavendra, K. Janardhan Reddy, eds), pp. 41–99. Springer, Dordrecht, the Netherlands.

Des Marais, D.L. & Juenger, T.E. 2010. Pleiotropy, plasticity, and the evolution of plant abiotic stress tolerance. *Ann. N. Y. Acad. Sci.* **1206**: 56–79.

Eallonardo, A.S., Leopold, D.J., Fridley, J.D. & Stella, J.C. 2013. Salinity tolerance and the decoupling of resource axis plant traits. *J. Veg. Sci.* **24**: 365–374.

Edwards, E.J. & Donoghue, M.J. 2013. Is it easy to move and easy to evolve? Evolutionary accessibility and adaptation *J. Exp. Bot.* **64**: 4047–4052.

Edwards, E.J. & Smith, S.A. 2010. Phylogenetic analyses reveal the shady history of C4 grasses. *Proc. Natl. Acad. Sci. USA* **107**: 2532–2537.

Edwards, E.J. & Still, C.J. 2008. Climate, phylogeny and the ecological distribution of C4 grasses. *Ecol. Lett.* **11**: 266–276.

Edwards, E.J., Still, C.J. & Donoghue, M.J. 2007. The relevance of phylogeny to studies of global change. *Trends Ecol. Evol.* **22**: 243–249.

Flowers, T.J. 2004. Improving crop salt tolerance. *J. Exp. Bot.* **55**: 307–319.

Flowers, T.J. & Flowers, S.A. 2005. Why does salinity pose such a difficult problem for plant breeders? *Agric. Water Manag.* **78**: 15–24.

Flowers, T. & Yeo, A. 1995. Breeding for salinity resistance in crop plants: where next? *Funct. Plant Biol.* **22**: 875–884.

Flowers, T.J., Galal, H.K. & Bromham, L. 2010. Evolution of halophytes: multiple origins of salt tolerance in land plants. *Funct. Plant Biol.* **37**: 604–612.

Furumoto, T., Yamaguchi, T., Ohshima-Ichie, Y., Nakamura, M., Tsuchida-Iwata, Y., Shimamura, M. *et al.* 2011. A plastidial sodium-dependent pyruvate transporter. *Nature* **476**: 472–475.

Glenn, E.P., Brown, J.J. & Blumwald, E. 1999. Salt tolerance and crop potential of halophytes. *Crit. Rev. Plant Sci.* **18**: 227–255.

Grass Phylogeny Working Group II 2012. New grass phylogeny resolves deep evolutionary relationships and discovers C4 origins. *New Phytol.* **193**: 304–312.

Hibberd, J., Sheehy, J. & Langdale, J. 2008. Using C4 photosynthesis to increase the yield of rice-rationale and feasibility. *Curr. Opin. Plant Biol.* **11**: 228.

Kadereit, G., Ackerly, D. & Pirie, M.D. 2012. A broader model for C4 photosynthesis evolution in plants inferred from the goosefoot family (Chenopodiaceae s.s.). *Proc. Roy. Soc. Lond. B* **279**: 3304–3311.

Kronzucker, H.J., Coskun, D., Schulze, L.M., Wong, J.R. & Britto, D.T. 2013. Sodium as nutrient and toxicant. *Plant Soil* **369**: 1–23.

Linder, H.P., Baeza, M., Barker, N.P., Galley, C., Humphreys, A.M., Lloyd, K.M. *et al.* 2010. A generic classification of the Danthonioideae (Poaceae). *Ann. Mo. Bot. Gard.* **97**: 306–364.

Maddison, W.P. & Maddison, D.R. 2006. Mesquite: a modular system for evolutionary analysis. pp., Version 2.72. http://mesquiteproject.org.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant, Cell Environ.* **25**: 239–250.

Munns, R. 2011. Plant adaptations to salt and water stress: differences and commonalities. *Plant responses to drought and salinity stress: developments in a post-genomic era*. *Adv. Bot. Res.* **57**: 1–32.

Osborne, C.P. & Freckleton, R.P. 2009. Ecological selection pressures for C4 photosynthesis in the grasses. *Proc. Roy. Soc. Lond. B* **276**: 1753.

Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. Roy. Soc. Lond. B* **255**: 37–45.

Pau, S., Edwards, E.J. & Still, C.J. 2012. Improving our understanding of environmental controls on the distribution of C3 and C4 grasses. *Glob. Change Biol.* **19**: 184–196.

Roy, S.J., Tucker, E.J. & Tester, M. 2011. Genetic analysis of abiotic stress tolerance in crops. *Curr. Opin. Plant Biol.* **14**: 232–239.

Rozema, J. & Flowers, T. 2008. Crops for a salinized world. *Science* **322**: 1478–1480.

Sage, R. 2001. Environmental and evolutionary preconditions for the origin and diversification of the C4 photosynthetic syndrome. *Plant Biol.* **3**: 202–213.

Sage, R.F. 2004. The evolution of C4 photosynthesis. *New Phytol.* **161**: 341–370.

Sage, R.F. & Monson, R.K. 1999. *C4 Plant Biology*. Academic Press, San Diego.

Sage, R.F. & Zhu, X.-G. 2011. Exploiting the engine of C4 photosynthesis. *J. Exp. Bot.* **62**: 2989–3000.

Sage, R.F., Sage, T.L. & Kocacinar, F. 2012. Photorespiration and the evolution of C4 photosynthesis. *Annu. Rev. Plant Biol.* **63**: 19–47.

Stromberg, C.A.E. 2011. Evolution of grasses and grassland ecosystems. *Annu. Rev. Earth Planet. Sci.* **39**: 517–544.

Subbarao, G., Ito, O., Berry, W. & Wheeler, R. 2003. Sodium, Äîa functional plant nutrient. *Crit. Rev. Plant Sci.* **22**: 391–416.

Taylor, S.H., Hulme, S.P., Rees, M., Ripley, B.S., Ian Woodward, F. & Osborne, C.P. 2010. Ecophysiological traits in C3 and C4 grasses: a phylogenetically controlled screening experiment. *New Phytol.* **185**: 780–791.

Zhu, X.Ä., Shan, L., Wang, Y. & Quick, W.P. 2010. C4 Rice: an ideal arena for systems biology research. *J. Integ. Plant Biol.* **52**: 762–770.

Received 18 October 2013; revised 13 December 2013; accepted 13 December 2013