Received: 8 April 2020 | Revised: 26 June 2020 | Accepted: 5 July 2020
DOI: 10.1111/pce.13857

REVIEW

Plant, Cell & Environment WILEY

Macroevolutionary and macroecological approaches to understanding the evolution of stress tolerance in plants

Lindell Bromham[1] | **Xia Hua**[1,2] | **Marcel Cardillo**[1]

[1]Macroevolution & Macroecology, Research School of Biology, Australian National University, Canberra, Australia

[2]Mathematical Sciences Institute, Australian National University, Canberra, Australia

Correspondence
Lindell Bromham, Macroevolution & Macroecology, Research School of Biology, Australian National University, Canberra ACT 0200, Australia.
Email: lindell.bromham@anu.edu.au

Funding information
Australian Research Council, Grant/Award Number: DP160103915

Abstract

Environmental stress response in plants has been studied using a wide range of approaches, from lab-based investigation of biochemistry and genetics, to glasshouse studies of physiology and growth rates, to field-based trials and ecological surveys. It is also possible to investigate the evolution of environmental stress responses using macroevolutionary and macroecological analyses, analysing data from many different species, providing a new perspective on the way that environmental stress shapes the evolution and distribution of biodiversity. “Macroevoeco” approaches can produce intriguing results and new ways of looking at old problems. In this review, we focus on studies using phylogenetic analysis to illuminate macroevolutionary patterns in the evolution of environmental stress tolerance in plants. We follow a particular thread from our own research—evolution of salt tolerance—as a case study that illustrates a macroevolutionary way of thinking that opens up a range of broader questions on the evolution of environmental stress tolerances. We consider some potential future applications of macroevolutionary and macroecological analyses to understanding how diverse groups of plants evolve in response to environmental stress, which may allow better prediction of current stress tolerance and a way of predicting the capacity of species to adapt to changing environmental stresses over time.

KEYWORDS
community assembly, diversification, model adequacy, model testing, phylogenetic analysis, salt tolerance, tippy

1 | A MACROEVOLUTIONARY VIEW OF STRESS TOLERANCE

Macroevolution is the study of patterns of variation in biodiversity over time, space and lineages, and macroecological analyses are used to test hypotheses about patterns of species diversity and distribution. While the evolution of stress response in plants is typically investigated at the level of genes, individuals or populations, taking a broader view can in some cases bring a different perspective to interesting puzzles, and offer a new way of tackling long-standing questions. In this review, we will consider some of the ways that macroevolutionary and macroecological analyses—“macroevoeco”—can shine a light on the evolution of environmental stress response in plants. This approach will rarely produce results with the kind of practical utility that plant physiologists, crop scientists or conservation biologists seek, but it may help us understand evolutionary dynamics and ecological patterns of stress tolerance. This review will focus on phylogenetic approaches to studying patterns of evolution of stress tolerance across angiosperm species.

There are many different ways that phylogenetic analyses can provide interesting perspectives on the evolution of stress tolerance. Firstly, phylogenetic analysis can be used to investigate the evolutionary lability of stress response syndromes: that is, how easily they can evolve in response to changing stresses or environmental shift. For example, estimates of phylogenetic lability have demonstrated that defence strategies against browsers can evolve rapidly and, therefore, can differ even between closely related species (Haak, Ballenger, & Moyle, 2014; Johnson, Ives, Ahern, & Salminen, 2014).

Secondly, investigations of functional associations between different traits can utilize phylogenetic methods to rule out association by phylogenetic inertia. Since species inherit many traits from their ancestors, related species will often have suites of the same traits not because the traits are functionally linked, but simply due to shared inheritance. For example, it has been proposed that plants must trade off herbivore tolerance (ability to grow back) against herbivore resistance (ability to limit damage), because any species should invest in one or other mechanism but not both (Pearse, Aguilar, Schroder, & Strauss, 2017). However, investment strategy might be strongly influenced by resource availability, with resistance being more strongly favoured in low resource environments. If species tend to inherit both their typical habitat (and thus likely resource availability) and their herbivore strategy from their ancestors, then it would be possible for these two traits to co-vary among species whether or not resource availability influences herbivore strategy. A phylogenetic analysis is needed to tease out any functional association beyond phylogenetic inertia (Pearse et al., 2017).

Thirdly, phylogenies can be used to detect changes in evolutionary dynamics over time in the evolution of stress responses associated with changing climate. For example, a phylogenetic study reconstructed the evolutionary origins of a post-fire recovery strategy, epicormic resprouting, in multiple lineages of Myrtaceae (Crisp, Burrows, Cook, Thornhill, & Bowman, 2011). Crisp et al. concluded that their molecular date estimates of early Paleogene origins of epicormic resprouting coincided with a biogeographic shift of these lineages into more fire-prone environments. They reported that resprouting and flammability of habitat showed a significant correlation on their phylogeny.

Fourthly, the macroevolutionary consequences of stress tolerance on long-term persistence and capacity for diversification can be examined. For example, adaptation to marginal soils has been implicated as a driver of diversification in some biodiversity hotspots, notably the South West Botanical Province in Australian and the Cape Floristic region in South Africa (Cowling, Rundel, Lamont, Arroyo, & Arianoutsou, 1996; Hopper & Gioia, 2004). However, it has also been suggested that specialisation to marginal soil types represents an "evolutionary dead-end" with many such species restricted to narrow endemism and unable to further diversify (Anacker & Strauss, 2014; Rajakaruna, 2018). These hypotheses can be formally contrasted using phylogenetic analyses (Day, Hua, & Bromham, 2016).

Fifth, phylogenies can be used to examine patterns of trait acquisition, in order to tease apart explanations for the existence of suites of traits associated with stress resistance. This may be useful for determining if the presence of some traits makes the evolution of other stress-related responses more likely. For example, evolution of C4 photosynthesis may aid the evolution of resistance to a range of stresses, including drought stress and salinity (Bromham & Bennett, 2014; Doubnerová & Ryšlavá, 2011; Osborne & Sack, 2012). However, it is important to note that these tests are dependent on the inference of ancestral states (Ackerly, Schwilk, & Webb, 2006), and influenced by the relative rates of gain and loss of different traits, so that caution must be applied in drawing conclusions about order of trait acquisition if one trait is gained and lost more frequently than the other (Bromham, 2016).

Sixth, phylogenies are increasingly being used to examine the influences of competition and environmental filtering on structuring ecological communities, which can be applied to understanding how environmental stress shapes community composition. For example, phylogenetic analysis has been used to ask whether competition is less important in structuring communities under dry, seasonal environmental conditions than in more permissive environmental conditions (Anderson, Shaw, & Olff, 2011). In woody plant communities of the Mediterranean Basin, phylogenetic patterns have been used to infer a stronger role for conserved traits in structuring communities in habitats with high fire frequency than those with low fire frequency (Verdu & Pausas, 2007).

These macroecological and macroevolutionary approaches are not perfect, and they trade off detailed knowledge of particular species or systems against a broader, general picture across many species, but they can provide a complementary view to more focussed investigations. The phylogenetic studies can then become a springboard for more detailed physiological investigations. Large-scale phylogenies are becoming widely available (e.g., Hinchliff et al., 2015; Smith & Brown, 2018), as are databases of species traits and distributions (e.g., Kattge et al., 2020; Webb & Donoghue, 2005), and most popular phylogenetic analysis packages are freely available. Of course, no phylogenetic tree is uncontroversial, and errors in phylogeny or inaccurate dates can impact macroevolutionary analyses (Duchêne, Hua, & Bromham, 2017; Heath, Zwickl, Kim, & Hillis, 2008; Stadler, 2012; Title & Rabosky, 2017). Furthermore, the relative density of taxon sampling can influence macroevolutionary inference (Heath et al., 2008; Hua & Lanfear, 2018), for example, by influencing the inferred number of trait gains and losses. But in most cases, using an imperfect phylogeny that gives an approximate indication of evolutionary history and relatedness will provide a better understanding of macroevolutionary patterns than analysing species traits without using any information on relatedness between species. In this review, we will focus on the evolution of salt tolerance in angiosperms as a fascinating case study that we have pursued in our own macroevolutionary and macroecological research, but the phylogenetic approaches we describe are applicable to a wide range of questions in the evolution of environmental stress response.

2 | THE HALOPHYTE PARADOX: A MACROEVOLUTIONARY PUZZLE

Halophytes are plants that are able to complete their life cycle under saline conditions that would prevent growth and/or reproduction in most species (Flowers & Colmer, 2008). Saline habitats are common and diverse, yet halophytes are comparatively rare. While 70% of the earth's surface is covered in salt water and up to 10% of terrestrial habitats are salt affected, less than 2% of flowering plant species are able to complete their life cycle in saline conditions (Flowers & Colmer, 2015). But equally striking as the rarity of halophytes is the diversity of lineages that have evolved salt tolerance. Salt-tolerant species have emerged in at least 100 angiosperm families (Santos, Al-Azzawi, Aronson, & Flowers, 2016), and even in some mosses and ferns (see Flowers, Galal, & Bromham, 2010).

The diversity of origins of salt tolerance in plants might reflect a diversity of mechanisms. Plants employ a range of adaptations to dealing with environmental salt, including structural features such as salt glands, physiological responses such as regulating water intake and output, anatomical responses such as compartmentalization of solutes, and biochemical adaptations such as up-regulation of ion transporters (Deinlein et al., 2014; Flowers & Colmer, 2008; Mudgal, Madaan, & Mudgal, 2010; Munns & Tester, 2008). Different species may employ different suites of traits, and often the precise mechanisms employed may be unknown or not fully characterised (Cheeseman, 1988). We should not be surprised if there are many different genes that can contribute to increased salt tolerance (Munns, 2005). Adaptation to salinity may involve modification of many existing biochemical pathways, regulatory networks or structural features, and might, therefore, evolve on a variety of different genetic backgrounds (Flowers & Colmer, 2008; Slama, Abdelly, Bouchereau, Flowers, & Savouré, 2015).

Yet despite the diversity of evolutionary origins of salt-tolerant plant lineages, and the variety of ways salt tolerance is achieved, most plant families contain relatively few salt-tolerant species (Table 1). For example, the angiosperm family, Amaranthaceae, contains more salt-tolerant species than any other family, yet only 16% of species in this family are listed as halophytes (according to the eHALOPH database: see Table 1). A small number of relatively species-poor families are dominated by halophytes, such as the marine sea grasses in Cymodoceaceae and Zosteraceae (Table 1), or the salt cedars in Tamaricaceae (Moray, Hua, & Bromham, 2015). But most families contain few or no halophytes (Flowers et al., 2010; Moray et al., 2015; Santos et al., 2016).

Furthermore, with a few exceptions, it has proved remarkably challenging to breed effective salt-tolerant crops, which are sorely needed given the increasing salinization of agricultural land globally (Rozema & Schat, 2013; Shabala, 2013; Shahbaz & Ashraf, 2013). Why, given that salt-tolerant habitats are widespread, are there relatively few naturally salt-tolerant species? And, given the successes in other aspects of crop improvement, why is it so hard to breed salt-tolerant crops, even though salt tolerance has evolved in many different plant lineages?

TABLE 1 Number of halophytes per family listed in the eHALOPH database (www.sussex.ac.uk/affiliates/halophytes/) for a sample of families containing the majority of salt-tolerant species

Family	Family size	Halophyte		Euhalophyte	
	Species	*Species*	*%*	*Species*	*%*
Amaranthaceae	2,052	353	17.2	55	2.7
Rhizophoraceae	142	22	15.5	22	15.5
Leguminosae	24,505	77	0.3	21	0.1
Poaceae	11,554	120	1.0	18	0.2
Zosteraceae	23	17	73.9	17	73.9
Cymodoceaceae	16	15	93.8	15	93.8
Malvaceae	4,465	29	0.6	15	0.3
Hydrocharitaceae	133	14	10.5	10	7.5
Arecaceae	2,522	26	1.0	10	0.4
Acanthaceae	3,947	14	0.4	10	0.3
Plumbaginaceae	635	59	9.3	8	1.3
Myrtaceae	5,970	21	0.4	8	0.1
Compositae	32,913	60	0.2	8	0.02
Lythraceae	604	9	1.5	7	1.2
Meliaceae	669	6	0.9	6	0.9
Aizoaceae	2,271	44	1.9	6	0.3
Potamogetonaceae	186	6	3.2	5	2.7
Primulaceae	2,788	8	0.3	5	0.2
Apocynaceae	5,556	8	0.1	4	0.1

Note: Halophytes are defined as being able to complete their life cycles at 7.8 dS/m, at least, or around 80 nM NaCl (Aronson, 1989). Euhalophytes are species with a high degree of salt tolerance that grow in seawater or tolerate >200 mM NaCl. This table is based on Santos et al. (2016), by permission of Oxford University Press.

One possible answer to the question of why there is a relative dearth of halophytes and limited number of salt-tolerant crops is that salt tolerance is a complex trait, involving a large number of genes and modification of many different anatomical structures and physiological processes, so that it is very difficult to evolve (Flowers & Flowers, 2005). Such a complex suite of traits may be a rare invention in the tree of life, and difficult to artificially select for given all of the interacting parts. The idea that salt tolerance is advantageous but rare because it is hard to evolve is essentially a macroevolutionary hypothesis, because it concerns the independent evolution of traits in many different lineages and their patterns of occurrence in time and space.

However, studies that have taken a macroevolutionary perspective cast doubt on the idea that salt tolerance is rare because it is hard to evolve. Although halophytes are rare, they are found in many different plant families. A few families have a large number of salt-tolerant species, but in most families, they are a tiny fraction of the known species (1% or less). This suggests that there have been multiple independent origins of salt tolerance in a diverse array of angiosperm lineages (Flowers et al., 2010). In other words, a large number of different kinds of plants have managed to adapt to saline conditions. To better understand the frequency of evolution of salt tolerance, we need to take a more detailed look at the number and pattern of evolutionary origins of salt tolerance, by mapping all salt-tolerant species on a phylogeny. When we mapped salt-tolerant species onto a phylogeny of grasses, we were surprised by what we saw (Figure 1). Instead of salt tolerance arising rarely then leading to a cluster of related salt-tolerant specialist taxa, halophytes show a "tippy" pattern: The salt-tolerant lineages occur mostly on or near the tips of the phylogeny, rather than the deeper internal branches (Bennett et al., 2013).

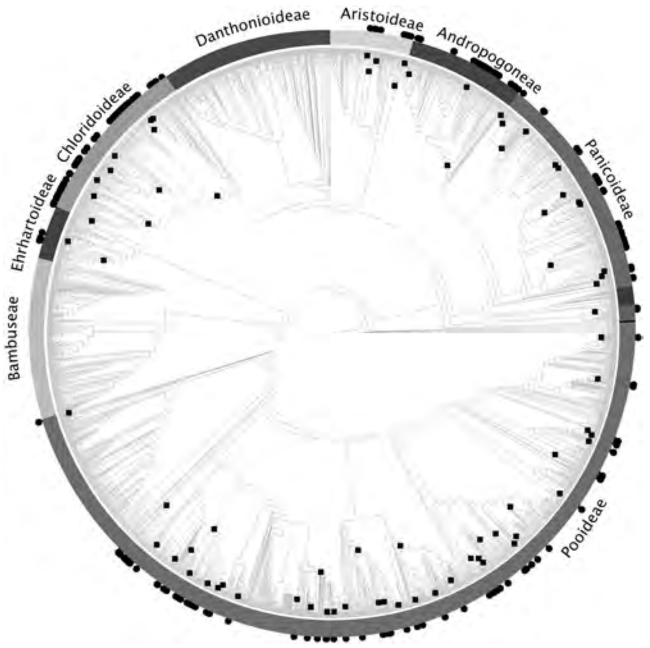

FIGURE 1 Phylogenetic distribution of salt tolerance in grasses. There are 200 known halophytes identified in this tree of 2,684 grasses (Edwards & Smith, 2010). The distribution of salt-tolerant species (marked with a black circle) is uneven, with some clades have few or no halophytes (e.g., Bambuseae) and some have many (e.g., Chloridoideae). But the striking pattern is that many salt-tolerant species occur in clades with few or no other halophytes, leading to the conclusion that there have been many independent origins of salt tolerance (marked with a black square). An alternative explanation would be that salt tolerance evolved fewer times but was lost in the majority of descendants. Reproduced from Bennett, Flowers, and Bromham (2013) with Permission from the Royal Society of London

Before we seek an explanation for this surprisingly tippy pattern, we first have to consider the potential impact of phylogenetic error: Could incorrectly inferred relationships lead to a false impression of tippiness across the tree? If two closely related halophytes are incorrectly placed in separate clades on the tree, it will falsely imply two origins of salt tolerance rather than one. But in this particular case study, the phylogeny would have to be seriously askew to account for the phylogenetic scatter. Most halophyte grass species occur in genera where there are few or no other known salt-tolerant species, so genus-level classification would need to be frequently misleading to explain this tippy pattern through phylogenetic error (Figure 1). Using different phylogenies gave similar results. An analysis based on a complete genus-level phylogeny of grasses (Bouchenak-Khelladi, Verboom, Savolainen, & Hodkinson, 2010) gave the same result as the molecular phylogeny of 2,684 species (Edwards & Smith, 2010).

Similarly, we also need to consider if incomplete data could generate a tippy pattern: If the salt tolerance status of many species was unknown, then it is possible that many of the relatives of known halophytes are actually salt tolerant but not marked as such in the phylogeny, inflating the phylogenetic isolation of halophytes. But given that there appear to be over 70 independent origins of salt tolerance on this grass phylogeny, and that there are multiple inferred origins in most of the subfamilies, there would have to be a vast number of "hidden halophytes" to fill in the gaps between known salt-tolerant species and reduce the number of implied evolutionary origins. For missing data to reduce the inferred number of origins to a sufficiently small number to match the "hard to evolve" explanation of halophyte rarity, the majority of members of many genera must be falsely classified as glycophytes (salt-sensitive species). Taxon sampling—the number of living species included in a phylogeny—could also affect the inferred number of origins of salt tolerance. If halophytes are more likely to be included in a phylogeny than non-salt-tolerant taxa, it will increase the relative number of inferred origins if salt tolerance is labile, but not if it is conserved (occurring in clades of related salt-tolerant taxa). If salt-tolerant taxa are less likely to be included in a phylogeny, it will reduce the inferred number of origins (if halophytes are phylogenetically scattered) or the number of inferred origins may be unaffected (if halophytes are phylogenetically clustered). Thus, it is difficult to predict the impact of incomplete taxon sampling on the inferred macroevolutionary patterns if we do not know how likely halophytes are to be included in a phylogeny. However, if we do know how many un-sampled taxa are halophytes, then the effect of incomplete taxon sampling can be explicitly accounted for by the SSE (State-dependent Speciation and Extinction) models (FitzJohn, Maddison, & Otto, 2009; Hua & Lanfear, 2018) or by simulations (Bromham, Hua, & Cardillo, 2016; Hua & Bromham, 2015) (see the next section).

Finally, it could be that this is just a weird grass thing, and not a feature of any other plant groups. However, a phylogenetic study of 22 angiosperm families (chosen because they had available phylogenies and sufficient numbers of known halophytes) revealed that half of those families had the same tippy pattern of salt tolerance as the grasses, including Asteraceae (with over 80 inferred origins of salt tolerance), Amaranthaceae and Cyperaceae (each with over 50 inferred origins: Moray et al., 2015). In these families, as in the grasses, most evolutionary origins of salt tolerance lead to only one or a few living halophyte species (e.g., see Figure 2). Of course, there are angiosperm families that show different patterns, such as the Tamaricaceae (tamarisk family), in which over half of the species are halophytes, all of which appear to arise from a single common origin of salt tolerance (Moray et al., 2015). But many of the families analysed showed the same remarkable tippy pattern, with many relatively recent evolutionary origins of salt tolerance, each of which led to only a few living halophytes.

3 | EVOLUTIONARY LABILITY: PHYLOGENETIC ESTIMATES OF RATES OF ORIGIN AND LOSS

Many angiosperm families show a tippy distribution of salt tolerance, with known halophytes scattered across the tree rather than grouped into clusters of salt-tolerant relatives, and it seems unlikely to be wholly explained by incomplete data or incorrect phylogenies (Moray et al., 2015). There are three possible macroevolutionary explanations of a tippy distribution on a phylogeny (Figure 3). One is that a recent environmental change has prompted the evolution of a similar trait in many different lineages at once. This has been offered as an explanation for the tippy distribution of fire resistance traits in woody plants on the Brazilian Cerrado (Simon et al., 2009), as a drying climate increased fire frequency and prompted parallel adaptation in many independent lineages. But parallel response to environmental change is unlikely to provide a convincing explanation for the tippiness of salt tolerance: Although salinity is increasing in many areas, naturally salt-affected habitats are as old as angiosperms. A second possible explanation for a tippy phylogenetic pattern is high trait lability: If a trait

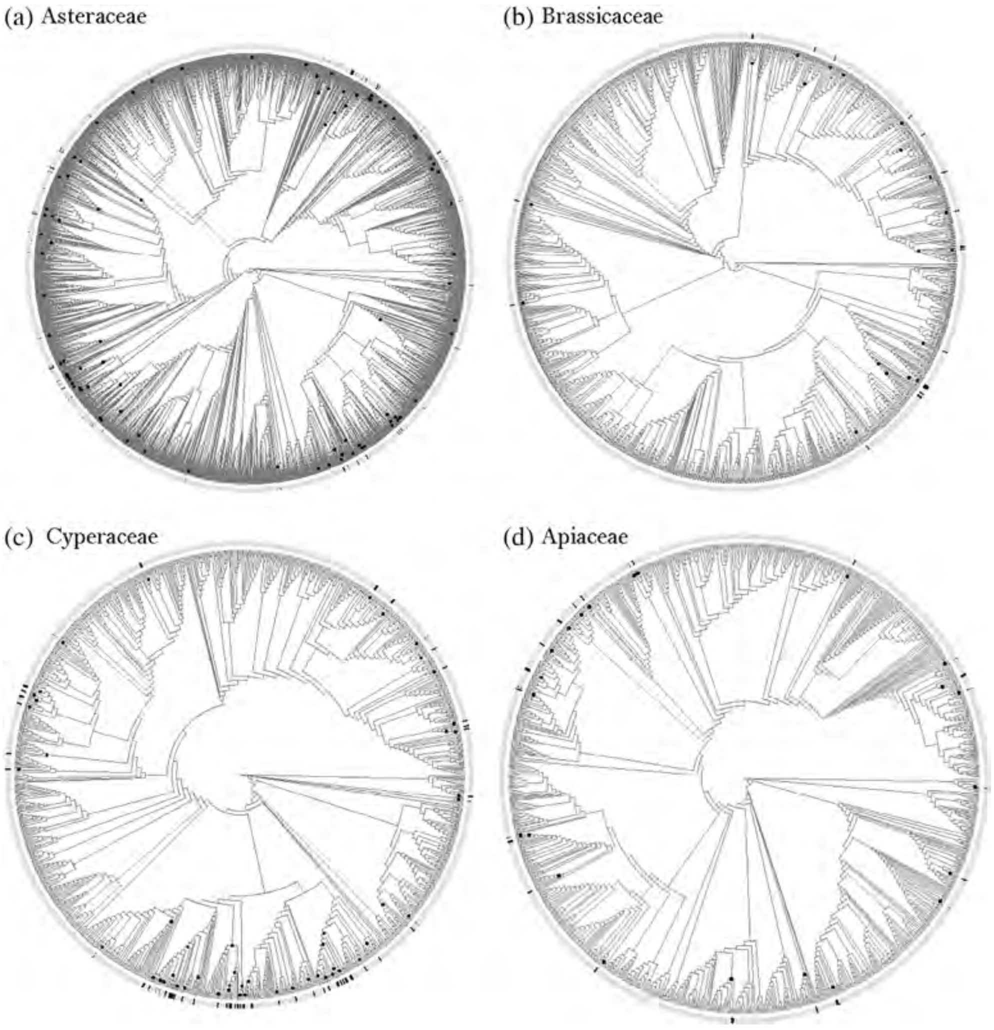

FIGURE 2 Examples of angiosperm families showing a “tippy” pattern of salt tolerance, where most halophytes have few close salt-tolerant relatives, implying a large number of independent origins of salt tolerance. Not all species are included in each phylogeny, and because of that not all known halophytes are mapped onto these trees, so the trees present the general pattern rather than a precise map of how many times salt tolerance has evolved. Reproduced from Moray et al., 2015 under Creative Commons 4.0 licence

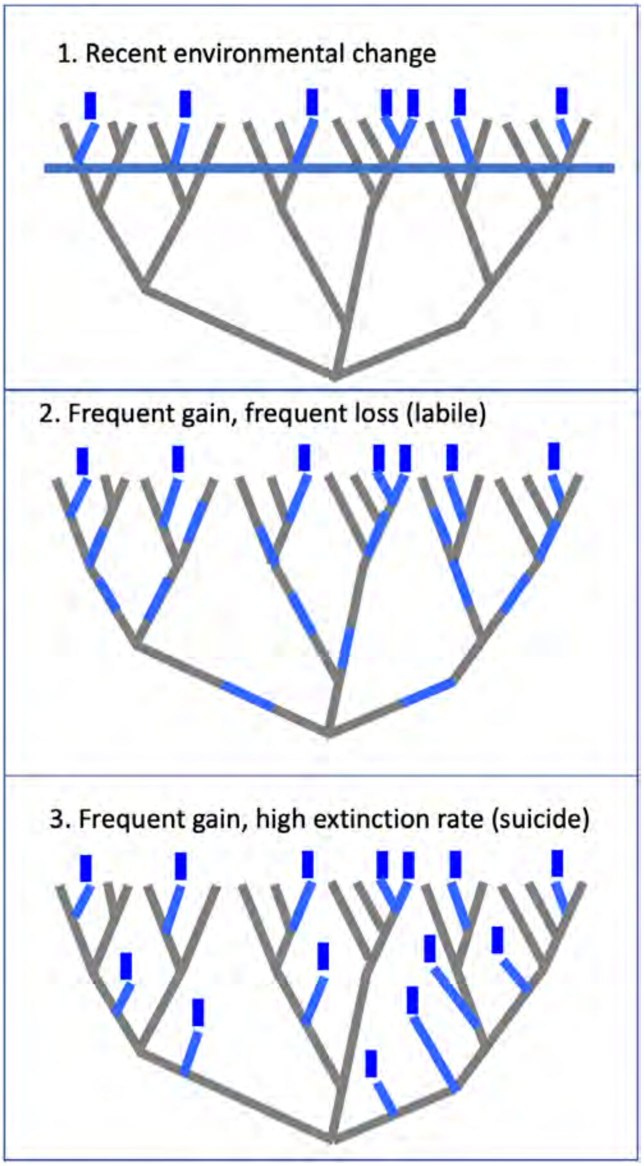

FIGURE 3 Schematic representation of three alternative explanations for a "tippy" distribution of a stress tolerance trait. The same distribution of the trait at the tips of the phylogeny (marked with blue blocks) could be produced by very different macroevolutionary mechanisms

changes frequently so that it is often gained and lost, then any observable instances of the trait will be the product of a recent gain (Figure 3). If a trait is highly labile, species will tend to be no more similar to their close relatives (with respect to this trait) than they are to distant relatives. For example, tolerance of herbivory in a group of *Streptanthus* (Brassicaceae) species is phylogenetically labile so that the species with high tolerance are scattered throughout the phylogeny, not clustered in related groups (Pearse et al., 2017). A third possible explanation is "macroevolutionary self-destruction": The trait is gained often but usually leads to the extinction of the lineage, such that any observable instances of the trait are recent origins that have not yet succumbed to extinction (Bromham et al., 2016). The classic example of macroevolutionary self-destruction is asexuality, which evolves frequently in many taxa due to the clear benefits to reproductive output and individual fitness, but rarely gives rise to stable long-term asexual lineages, nor to diverse clades of asexual species (Maynard Smith, 1978; Williams, 1975).

In macroevolutionary terms, a tippy distribution could be interpreted as evidence for lineage selection. While natural selection is typically described in terms of differential survival and reproduction of individuals leading to the promotion of advantageous genetic variants, the same process applies to any level of organisation where heritable variants influence their own chance of survival and reproduction. Evolutionary lineages can differ in features that influence their long-term survival or their speciation rate, and those traits can be heritable in the sense that descendant species will tend to resemble their immediate ancestors. We can recognize positive lineage selection when certain traits, such as pollination modes, lead to greater evolutionary persistence or higher rates of speciation and, therefore, tend be found in more species (Serrano-Serrano, Rolland, Clark, Salamin, & Perret, 2017). Conversely, we can consider cases of negative lineage selection if some traits, such as selfing, lead to higher extinction rate or lower speciation rates and are, therefore, found in relatively fewer descendants (Goldberg et al., 2010). We might be able to recognize cases of lineage-level selection from phylogenetic distributions of traits. For example, some cases of tippy distribution of pollinator specialisation have been interpreted as a sign of decreased speciation rate. Cases have been identified where lineages with moth pollinated flowers, self-pollinated flowers or red flowers appear not to diversify at the same rate as other species, so on the phylogeny they occur as "lonely species" rather than in clades of species all sharing the same pollination syndrome or flower type (Day et al., 2016; Gamisch, Fischer, & Comes, 2015; Ng & Smith, 2018; Tripp & Manos, 2008). A similar argument has been made for the tippy distribution of asexuality, especially in animals, where it arises often but rarely gives rise to long-lived clonal species, nor to diverse clades of asexually reproducing species (Schwander & Crespi, 2008).

What can we learn about the evolution of stress tolerance from "tippy" phylogenetic distributions? The first thing to note is that all three possible macroevolutionary explanations for tippy traits suggest that the trait of interest is gained often in many different lineages. The recent environmental change explanation (Figure 3a) suggests that many different lineages can all respond to a particular environmental stress by evolving similar traits. The labile explanation (Figure 3b) suggests that the trait is gained and lost often enough that many more lineages have acquired the trait than are currently observed, because few lineages have maintained the trait over long time periods. The self-destructive explanation (Figure 3c) suggests that the trait emerges surprisingly often but its rarity is due to its inability to persist. Therefore, given the observed tippy distribution of salt tolerance, we can conclude that it is not "hard to evolve". Instead, salt tolerance arises often in a wide range of flowering plant lineages, presumably due to the competitive advantage of being able to persist in saline conditions where other salt-sensitive species cannot grow.

If salt tolerance evolves often but halophytes are rare, then there must be longer-term costs to salt tolerance that prevent it from persisting over longer evolutionary timescales. The tippy distribution of salt tolerance might suggest that short-term benefits of acquiring salt tolerance in terms of a competitive advantage are countered by long-term disadvantages, due to the cost of investment in salt tolerance mechanisms compromising the ability to maintain viable populations or cope with other environmental or biological stresses. But simply noting a tippy distribution is not sufficient to confirm this explanation. We need to move beyond pattern recognition and into hypothesis testing. To do this, we must show that the tippy distribution could not have occurred by chance and that it is consistent with the expectations of high gain and high loss or rapid extinction. We need four macroevolutionary tools to do this: We need a metric (to be able to describe the degree of tippiness of a trait in an objective and quantitative way), a null model (to ask if the pattern we see could have occurred by chance), model tests (to evaluate whether the hypothesis of macroevolutionary self-destruction of halophytes provides a better explanation of the phylogenetic pattern than alternative explanations) and tests of model adequacy (to reassure ourselves that these macroevolutionary processes really could produce the pattern we see).

There are several possible measures of the tippiness of a trait (Agnarsson, Avilés, Coddington, & Maddison, 2006; Bromham et al., 2016; Ng & Smith, 2018; Schwander & Crespi, 2008). The simplest and most effective is the number of species per origin, that is, the number of species with the trait divided by the number of inferred origins. Origins could be estimated using ancestral state reconstruction but a simple and conservative approach is using a parsimony principle—what is the minimum number of origins we have to infer to explain the observed pattern? If there are many species with the trait but a low number of tips per origin then the trait evolves often but does not give rise to many descendants with the trait. How do we decide when the number of tips per origin (NoTO) is the sign of a trait that has short-term benefits but long-term macroevolutionary costs? We need a null model that tells us what values of NoTO to expect if there is no lineage selection going on. In other words, we need a distribution of possible values of NoTO for our phylogeny if the trait has no effect on speciation, extinction or loss rates and is simply evolving by a random walk (i.e., equal chance of being gained on any lineage at any point in time). In this case, we would expect the evolutionary origins of the trait to be scattered throughout the tree, defining clades of different sizes—some near the tips, some deeper in the tree. There would be a distribution of a number of tips per origin, from older origins, defining large clades with lots of halophytes, to recent origins near the tips leading to only one or two species.

To generate a suitable phylogenetic null distribution that represents the expected number of tips per origin in the absence of any influence of salt tolerance on speciation or extinction, we need to generate possible phylogenies by simulating an evolutionary process in which all lineages have the same chance of speciating, going extinct and acquiring salt tolerance throughout their history. The simulated trees need to have the same number of halophyte and glycophyte species as our real dataset does. This can be done efficiently by simulating trees backwards, starting from tip species and joining two lineages at a time until we reach the root (Hua & Bromham, 2015). Then, we can compare the observed number of tips per origin (NoTO) from our real data to the null distribution we have generated and ask how likely is it that we would have observed the pattern in the real data given the null model of no effect of salt tolerance on speciation, extinction or trait loss. When we applied this method to a phylogeny of the grass subfamily, Pooideae, containing 461 species, including 24 halophytes, there was an average of 1.1 species from each inferred origin, which is significantly fewer than expected (2.6 ± 1.1) under the null model (Bromham et al., 2016). Note that the value of NoTO reflects phylogenetic sampling not total number of species for an incomplete phylogeny (i.e., one that does not include all described species in the clade).

Now that we have established that the pattern of salt tolerance in the Poiodeae is not what we would expect if the trait has no effect on speciation, extinction and loss, we can compare the explanatory power of different macroevolutionary models for the tippy distribution. The most common framework for these macroevolutionary models belong to the SSE (State-dependent Speciation and Extinction) family of models, where lineage speciation or extinction rates depend on lineage trait and the trait can have different gain and loss rates (Maddison, Midford, & Otto, 2007). Maximum likelihood is used to compare the relative fit of alternative models to the observed data, particularly models where trait gain or loss rates or lineage speciation or extinction rates vary over the phylogeny. In this case, we can ask if a model that has higher loss rates or higher extinction rates associated with salt tolerance provides a more plausible explanation of the observed phylogenetic distribution of halophytes than the null model (Bromham et al., 2016). This can be done by any standard model comparison approach, such as a likelihood ratio test.

However, it is important to recognize that these model tests tell you which of a set of candidate models provide the best fit to the data, but there is no guarantee that the "best" model describes the data at all well. SSE models provide the best solutions to the parameter values of the candidate models and reject some as a poorer fit than others, but they have two major limitations in hypothesis testing: They cannot tell you whether the best fit model is a plausible explanation of your data, and power to detect diversification rate dynamics is dependent on sample size and trait distribution (e.g., Davis, Midford, & Maddison, 2013; Gamisch, 2016). How can we tell if any of our macroevolutionary models provide plausible descriptions of our data? We need to add a test of model adequacy that asks if our observed data could have been generated if this model were true. Simulations can provide a distribution of values of the tippiness metric under different candidate models to check whether the observed metric falls within the possible values generated by that model for a dataset of comparable size (Hua & Bromham, 2015). If not, then the model can be rejected as an inadequate description of the process that produced the real data. Using this approach, we identified the most likely explanations of the tippy pattern of salt tolerance in Pooideae as either high rate of gain with even higher rate of loss, or high rates of gain and loss coupled with high extinction rate (Bromham et al., 2016).

4 | STRESS TOLERANCE SYNDROMES: CONNECTING TRAITS THROUGH PHYLOGENETIC ANALYSIS

The tippy phylogenetic pattern of salt tolerance suggests that it is surprisingly easy to gain in a wide variety of lineages, although prone to loss through reversal or extinction. One possible explanation for the frequent gain of salt tolerance is that it evolves by modification of many existing pathways and structures, so that it represents many small tweaks to physiology, rather than a giant leap requiring the simultaneous acquisition of large changes to structures and processes. If this is the case, then some enabling traits may represent a stepping stone on the way to salt tolerance, such that possessing particular traits might make evolving salt tolerance easier. We can use phylogenetic analysis to ask whether some traits increase the chances of evolving salt tolerance.

For example, it has been noted that salt tolerance is more likely to occur in C4 grasses than C3 grasses (Eallonardo, Leopold, Fridley, & Stella, 2013). But C4 grasses are clustered on the phylogeny, all occurring in the PACMAD clade that contains about half of all grass species. If PACMAD lineages share other features that also increase the chance of evolving salt tolerance, for example, if they tend to occur in regions more likely to contain saline habitats (Edwards & Smith, 2010), this could generate an incidental association between C4 photosynthesis and salt tolerance, because more salt-tolerant species would fall within a group that also contains more C4 species. To examine the association, we can plot the origins of both C4 and salt tolerance on the phylogeny, and ask if salt tolerance evolves more often in C4 lineages than in C3 lineages. First, we need to describe how many halophytes we expect to occur in C4 lineages by chance if there is no functional connection between photosynthetic mode and evolution of salt tolerance. One way to do this is to do a "tip shuffle" randomization, where the number of known halophytes is randomly assigned to tips of the phylogeny, then the procedure repeated many times, to derive a null distribution of the expected number of halophytes in every clade if they occur completely at random with respect to evolutionary relationships. A tip shuffle gives you possible distributions of a trait if there is no phylogenetic signal in the trait, that is, if close relatives are no more likely to share the trait than more distant relatives. In this case, if salt tolerance was just as likely to occur in any lineage, irrespective of whether they were C3 or C4, then we would expect around 20 halophytes to occur in C3 PACMAD grasses. Instead, there were only three C3 halophytes in this group (Bromham & Bennett, 2014).

But a tip shuffle is usually a poor null model for an evolutionary hypothesis because it assumes there is no heritability of traits at the phylogenetic level. A more reliable test of association between traits takes the phylogeny into account by asking whether one trait is more likely to evolve in a lineage that has already acquired (or inherited) another trait. A macroevolutionary model in which salt tolerance is linked to C4 provides a significantly better fit to the data for the Poaceae as a whole, and within the PACMAD clade, where the estimated rate of transition from glycophyte to halophyte is seven times higher in C4 lineages than in C3 lineages (Bromham & Bennett, 2014). These results suggest that having C4 photosynthesis greatly increases the rate of evolving salt tolerance, possibly because it reduces transpiration and increases water-use efficiency (Taylor et al., 2010), although we cannot rule out the alternative explanation that C4 is more likely to arise in lineages that occur in saline environments. In this sense, C4 photosynthesis may represent an enabling trait that provides a platform for the development of greater stress tolerance. The association between enabling traits and evolution of environmental stress tolerance (Christin et al., 2013) raises the possibility that we may have more success in increasing salt tolerance in species or varieties that already have enabling traits (Flowers & Flowers, 2005).

Phylogenetic analysis could also be applied to investigating the prevalence of cross-tolerances. The effects of particular physiological mechanisms on multiple tolerances are usually studied within specific species, for example, by examining how the accumulation of osmolytes under salt stress can increase resistance to oxidative damage drought stress or frost (Puniran-Hartley, Hartley, Shabala, & Shabala, 2014), or how wound response can trigger pathways that increase cross tolerance to a range of environmental stresses including salinity (Bowler & Fluhr, 2000; Capiati, País, & Téllez-Iñón, 2006). But we can also take a macroevolutionary view of cross tolerance by asking whether lineages with a known ability to tolerate one form of environmental stress also have increased resistance to other stresses. This can be done using published information on plant traits. For example, when we combined the lists of known halophytes and hyper-accumulators, we found that there are more species of angiosperms that can tolerate both salinity and heavy metals than would be expected by chance given the rarity of both of those traits (Moray, Goolsby, & Bromham, 2016). Cross tolerance can also be inferred from distribution data. For example, distribution data for Australian *Acacia* species were compared to predicted soil salinity and pH, then species with high inferred tolerance were plotted on a phylogeny (Bui, Thornhill, & Miller, 2014). Phylogenetic analysis suggested that these two traits co-occurred more than would be expected by chance.

While a phylogenetic investigation of cross-tolerance lacks the detail of a more focussed physiological study, it has the advantage of revealing generalities and avoiding over-interpreting incidental associations within key species. Another advantage of this macroevolutionary approach is that it can identify tolerances that are associated in relatives, even when those tolerances do not co-occur in the same species. For example, angiosperm families that contain halophytes are also more likely to contain species that can hyper-accumulate heavy metals (Moray et al., 2016), which might suggest that the same enabling traits can aid the evolution of both salt tolerance and heavy metal tolerance.

More generally, we might be able to use phylogenetic correlations to distinguish different kinds of co-association between stress tolerances, which we could test using phylogenetic correlations between stress resistance traits:

1. Supertolerators: Species with one kind of tolerance are more likely to also tolerate other stresses (the correlation coefficient should

be close to one because multiple tolerances occur in the same taxa, so different tolerances have similar phylogenetic distributions).
2. Tolerance syndrome: Lineages that give rise to species with one kind of tolerance are more likely to give rise to species with other tolerances, potentially because enabling traits provide a basis for developing tolerance to a range of stresses (the correlation coefficient should be significantly positive, but lower than 1 because different kinds of tolerance occur on different taxa that share a common ancestor).
3. Specialist tolerators: Lineages with investment in one kind of tolerance may be less able to adapt to other stresses, suggesting that some combinations of stresses are difficult to tolerate in concert (the correlation coefficient should be significantly negative, because having one tolerance reduces the chances of having another tolerance).
4. No association: Tolerances vary over the tree without significant co-occurrence beyond phylogenetic inertia (requires an explicit phylogenetic null model to test).

It is important to note that spatial co-occurrence is a potential confounding factor in these analyses. For example, the relationship between C4 and salt tolerance in grasses could be an incidental result of biased habitat selection. C4 plants may be more likely to be found in open or arid areas (Edwards & Smith, 2010; Liu, Edwards, Freckleton, & Osborne, 2012), which may also be more likely to be salt affected than closed canopy or moist environments (Bromham & Bennett, 2014). Similarly, the co-occurrence of salt-tolerance and alkalinity-tolerance in *Acacia* species may be because both saline and alkaline soils tend to occur in arid parts of Australia, rather than explained by the possession of traits that predispose species to deal with both alkalinity and salinity (Bui et al., 2014). A spatially explicit analysis that used distribution data could be used to tease this co-variation apart (see below).

5 | STRESS TOLERANCE IN SPACE AND TIME: SPECIES ASSEMBLAGE FORMATION UNDER HARSH CONDITIONS

We have seen how a macroevolutionary approach to stress tolerance can explore issues such as how "evolvable" stress tolerance is, whether particular suites of traits help or hinder the acquisition of stress tolerance, and the long-term evolutionary consequence of adapting to environmental stress (such as increased extinction rate). A macroecological approach is complementary to this, and allows us to ask questions about the formation of ecological communities in areas of environmental stress. When we take a macroecological approach to understand the evolution of tolerance to environmental stress, we can make use of the information we can gain from analysing species spatial distribution data, which are widely available for many plant groups and regions. At the simplest level, we can use distribution data, combined with information, on the environmental conditions at these locations to predict environmental tolerances. So, for example, the present data can be used to identify salt-tolerant species on the assumption that a species that is found consistently in areas with high salinity must be salt tolerant (Bui et al., 2014; Saslis-Lagoudakis, Hua, Bui, Moray, & Bromham, 2015).

We can also use spatial distribution data, combined with phylogenies, to compare models for the formation of species assemblages in areas of high environmental stress. Here, we consider three alternative models (Table 2):

1. Specialists colonize: If stress tolerance is difficult to evolve and comes at the cost of being less competitive in normal conditions, then we expect areas of high stress to be primarily occupied by specialist tolerator taxa, which are unable to persist in unaffected areas, and these tolerator taxa will tend to descend from relatively few common ancestors. If this is the case, then species assemblages in areas of high stress will not be a random sample of lineages from the surrounding region, because they will tend to be colonized by specialists that disperse from other areas of high stress. Therefore, species found in high-stress locations will tend to be more closely related to those in other similar extreme habitats than they are to the species in the surrounding areas.
2. Locals adapt: If stress tolerance is easy to evolve, then it is possible that areas of extreme conditions will be colonized by lineages from the surrounding region that can adapt to extreme conditions. If this is the case, then species assemblages in areas of high stress will be a sub-sample of lineages in the surrounding area, so will not show a significantly different pattern of phylogenetic clustering from species in the surrounding area, and species in high-stress locations will tend to be more closely related to species in the surrounding areas than those in other similar extreme habitats.
3. Generalists disperse: If the evolution of stress tolerance does not come at the cost of competitive ability in normal conditions, then we might expect the evolution of generalist lineages that have wide geographic distribution, across areas of both high and low stress. In this case, species found in areas of high stress will be these generalists, not just a random sub-sample of lineages in the surrounding area. But our predictions will depend on whether these generalists have different phylogenetic distribution from the other species. If these generalists evolve rarely, then they will show the same phylogenetic patterns as specialists (Table 2). If generalists can persist in areas of both high and low stress, then we might not expect significantly different phylogenetic distance between species assemblages in areas of low and high stress, or between species assemblages in nearby areas or distant areas.

To test the three alternative models, we need to identify species occurring in areas of high stress and compare them to species in the regional pool, that is, species in the area that we presume would have the ability to disperse into the high-stress area, if they can tolerate the conditions. This can be done using species distribution models (SDMs) which use a species current distribution to predict occurrence based on environmental conditions (Araújo & Pearson, 2005). However, we cannot rule out that species are absent from an area not

TABLE 2 Macroecological models for the formation of species assemblages in areas of high environmental stress, and predictions that they make on the phylogenetic distribution of species with high levels of tolerance to a particular environmental stress, as well as predictions of the overall levels of relatedness between assemblages in dispersed high-stress areas compared to species in the surrounding (low-stress) areas

Model	Process	Phylogenetic distribution	Assemblage structure	MPD or MNTD	Assemblage similarity	phyloBeta
Specialists colonize	Tolerance evolves rarely, disperse to high-stress environments	Tolerators clustered	Species in high-stress environments more closely related to each other than to species in surrounding areas	Lower than null distribution	Species in areas of high stress are more closely related to species in other high-stress areas than surrounding areas of low-stress	Positively correlated with stress
Locals adapt	Tolerance evolves often, many species adapt to areas of stress	Tolerators no different from non-tolerators	Species in high-stress environments not more or less closely related to each other than to species in surrounding areas	Do not differ from null distribution	Species in areas of high stress are more closely related to species in surrounding low-stress areas than in distant areas	Positively correlated with distance
Generalists disperse (1)	Tolerance evolves rarely, generating generalists that are found in both high and low stress areas	Tolerators clustered	Species in high-stress environments more closely related to each other than to species in surrounding areas	Lower than null distribution	Species in areas of high-stress are more closely related to species in other areas of high-stress than to surrounding areas of low stress	Positively correlated with stress
Generalists disperse (2)	Tolerance evolves often, generating generalists that are found in both high and low stress areas	Tolerators scattered	Species in high-stress environments less closely related to each other than to species in surrounding areas	Higher than null distribution	Species in two areas won't differ in phylogenetic distance if generalists are phylogenetically scattered and widely disperse	Not correlated with stress or distance

Note: These two predictions can be examined using values of MPD or MNTD, and by a regression of values of phylobeta against both distance and environmental gradients.

because they are unable to tolerate the conditions but because they are unable to disperse to the area or could not compete with the incumbent species. New developments of SDMs have started accounting for these biotic factors that also restrict species distribution. The simplest model is to add a variable to the traditional correlative SDM model, in addition to environmental factors, that describes dispersal cost, then to estimate residual covariance between different species to account for biotic interactions (Miller & Holloway, 2015). The most complicated model is to explicitly model population dynamics of different species, while linking demographic rates to environmental factors (Zurell, 2017).

Once we have a list of species that are present and absent in areas of high stress and in the surrounding areas, we can compare the observations to predictions about the phylogenetic distribution of tolerators in areas of high stress, specifically whether tolerators tend to be more closely related to each other than they are to taxa in the surrounding regions (Table 2). This can be explored using widely used phylogenetic metrics: The mean pairwise phylogenetic distance between species in a species assemblage (MPD) and the mean nearest taxon phylogenetic distance (MNTD) (Webb, Ackerly, McPeek, & Donoghue, 2002). Given a phylogeny, these metrics can be calculated for the observed species assemblage in an area of high stress. MPD is the average phylogenetic distance between members of an assemblage, so it is calculated by summing all intervening branch lengths between any pair of species in the assemblage, then taking the mean path distance across all pairs. A low value suggests that an assemblage consists of close relatives, and a higher value suggests they are more distantly related to each other. MNTD is similar but instead of taking the average path length across all possible pairs of species in the assemblage, it uses only the phylogenetic distance from each species in the assemblage to the most closely related species in the assemblage. To generate a null distribution to compare this observed value, we can randomly sample species from the pool of species (those that have the ability to disperse to the area of high stress regardless of whether they could tolerate those conditions).

Now we can compare the observed metric (how closely related are species found in high stress areas?) to the null distribution (how closely related are random samples of the same number of species from the regional pool?). If the observed value is significantly smaller than the null distribution, we conclude that species in areas of high stress are more closely related on the phylogeny than a random sample of species that can disperse to these areas. If the species assemblage in areas of high stress has similar phylogenetic distance to a random sample of species that can disperse to these areas, this is consistent with tolerator taxa evolving from many local lineages evolving stress tolerance (Table 2).

We can test predictions about the composition of species between two species assemblages using various measures of phylogenetic distance, which are referred to as phylogenetic beta diversity metrics (Graham & Fine, 2008). Phylogenetic beta diversity not only accounts for dissimilarity in species composition between assemblages, but also the overall phylogenetic distance between assemblages. The aim of phylogenetic beta diversity measures is to capture the processes structuring communities. For example, by assuming that closely related species will have more similar suites of traits and are more likely to compete with each other, comparisons of the relative degree of relatedness within and between communities have be used to test the role of competition in shaping community assemblages (Graham & Fine, 2008), or to contrast the relative role of environmental difference and spatial distance in shaping similarity among species assemblages (Swenson, 2011). There are different measures of phylogenetic beta diversity, but they are all derived from the taxonomic-based beta diversity (for example, the Sorenson dissimilarity index) by replacing species richness with phylogenetic diversity, which is the sum of intervening branch lengths connecting all the species (Leprieur et al., 2012).

We can use phylogenetic beta diversity to ask whether the species found in high-stress environments tend to be more closely related to each other than they are to the species found in the surrounding, low-stress areas using an approach called phylogenetic generalized dissimilarity modelling (Ferrier, Manion, Elith, & Richardson, 2007). This is basically a regression model, with phylogenetic beta diversity between assemblages in two areas as the response variable and the geographic distance and the difference in level of environmental stress between the two areas as independent variables. This model does not need to assume a linear relationship between geographic and phylogenetic distance, because a wide range of possible functions can be fitted. If the difference in stress level between two assemblages has a significantly positive regression coefficient against the measure of phylogenetic beta diversity, this suggests that species in areas of high stress tend to be more closely related to species found in other high-stress areas than to species found in low-stress areas, given the same geographic distance between the two areas. This result would support the “specialists colonize” model, or the “generalists disperse” model if general tolerance to both high- and low-stress evolves rarely. If the geographic distance between two assemblages has a significantly positive regression coefficient against phylogenetic beta diversity, this supports the “locals adapt” model, because it suggests that the species in nearby areas, regardless of their difference in stress level, are more closely related to each other than they are to more distant species, consistent with adaptation of local species to areas of high stress rather than colonization of stress-tolerant specialists. If neither the stress level between two assemblages nor the geographic distance between two assemblages have a significant regression coefficient against phylogenetic beta diversity, this suggests that patterns of relatedness between two areas are much the same whether we are comparing areas that differ in stress level or are the same, or whether we compare nearby or distance areas. This result suggests that acquisition of tolerance to high stresses is not rare or phylogenetically clustered and that most species that acquire tolerance are able to disperse across both high- and low-stress areas. This pattern is consistent with the “generalist disperse” model when generalists evolve frequently so are phylogenetically scattered (Table 2).

6 | FILLING IN THE GAPS: MACROECOLOGICAL PREDICTIONS OF STRESS TOLERANCE

One of the common limitations of macroevolutionary or macroecological studies of environmental stress tolerance is that we have incomplete knowledge of which species have high tolerance. While some species are well known to tolerate harsh conditions, for many taxonomic groups it is likely that there may be other species whose tolerance levels have not been fully characterised that may be capable of persisting in the same conditions. For example, published lists of known halophytes almost certainly underestimate the number and diversity of species able to grow in saline conditions (Flowers et al., 2010). Incomplete lists of tolerators may increase the scatter of tolerators on the phylogeny and, therefore, influence inferred macroevolutionary patterns (Saslis-Lagoudakis et al., 2015). One potential remedy for this problem is to use macroecological analyses to predict the tolerance level of all species. Because “stress” is defined with respect to the conditions that a population of a particular organism can tolerate (Bijlsma & Loeschcke, 2005), we can use the distribution of conditions under which a population is found to characterise its current tolerance limits.

Given that distribution data are typically incomplete, consisting of reported specimens from particular locations but limited information on un-sampled areas, we need to be able to use patchy information to infer the likely tolerance range of a species. Species distribution models (SDM) allow us to use available distribution data to characterise the range of conditions under which a species can persist (Peterson et al., 2011). But we can improve our prediction of species tolerance to environmental stress if we incorporate evolutionary history into our SDMs. Incorporating phylogeny into SDMs allows us to model the impact of evolutionary processes on current distribution and leads to better prediction of salt-tolerant species (Hua, Cardillo, & Bromham, 2019).

We have applied this approach to studying the evolution of salt tolerance in Australian *Acacia* species (Hua et al., 2019). The approach not only better predicts known *Acacia* halophytes than using SDMs alone, but also paints a picture of the history of salt tolerance in Australian *Acacia* species over time and space. Over time, there are three peaks in the amount of adaptation to salt tolerance per lineage, with the highest peak near the present and two additional lower peaks around 10 and 17 Ma. Over space, lineages that are currently distributed in areas with high salinity are inferred to have undergone more adaptation events to evolve salt tolerance. In particular, salt-tolerant lineages in two recent radiations have the highest inferred rate of adaptation to salt tolerance. Combined with the result that lineages with high occurrence of speciation events tend to also have high occurrence of adaptation, this study suggests a potentially important connection between speciation and the evolution of salt tolerance in *Acacia*. The prevalence of speciation in *Acacia* species along salinity gradients in the environment may provide a mechanism for the high lability of salt tolerance (Bromham et al., 2016).

Joint modelling of lineage evolution, dispersal and adaptation to environmental differences provides a method for comparing the relative degree of past evolutionary change in lineages in response to changes in environmental stress. The lineage-specific patterns of "evolvability" in response to stress might then provide a basis for predicting a lineage's likely adaptability to increasing environmental stress (Hua et al., 2019), on the assumption that a lineage that has demonstrated evolutionary lability in the past may continue to do so in future. However, continued adaptation to increasing environmental stress may not be possible if the lineage has already reached the limit of available variation in that trait. Conceivably, we might be able to use the shape of the distribution of conditions in which a species is currently found to predict the adaptation limit of a species clade (Figure 4). The shape of this curve may help to define apparent limits to adaptation for the species. If there are environmental conditions in the distribution range of a specific species that are above this limit, then we expect a drop in the abundance of the species in areas with environmental conditions above the limit (Figure 4b). The sharper the drop in abundance at the edge of the distribution, the closer the species is to its upper limit on adaptation to stressful conditions. Plotting the median value of the distribution against the skew may reveal the extent to which a group of species has reached an adaptation limit for that clade. If a clade is reaching its adaptation limit, then we expect that, for the species within the clade, the skewness of a species distribution should be positively correlated with the mode of the species distribution (Figure 4c). In this way, we may be able to identify cases where a clade has reached an upper bound on climatic or edaphic conditions appears, suggesting it may lack the genetic variation that would underpin the capacity to respond to a shift in conditions beyond current limits. This kind of approach may provide a useful perspective on the potential for species to adapt to shifting conditions under climate change.

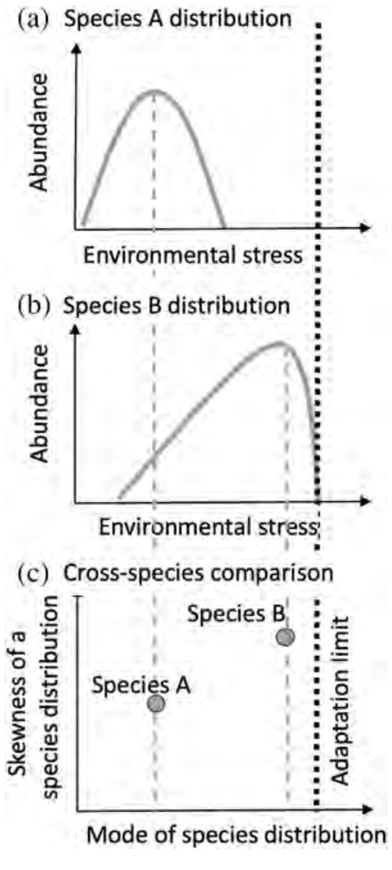

FIGURE 4 Schematic representation of the relationship between the mode and the skewness of a species distribution for species that share the same adaptation limit to physical tolerance (e.g., because they are close relatives with similar physiology or co-habitants with similar environmental adaptations), although one is distributed in non-stressful conditions (a) and one is at the limit of environmental stress tolerance (b). Collectively, these species should reflect a positive relationship between the mode and the skewness of species distribution in the clade (c) [Colour figure can be viewed at wileyonlinelibrary.com]

7 | CONCLUSION

In this review, we have considered some of the ways that a macroevolutionary and macroecological perspective can provide an insight into the evolution of environmental stress responses in plants. This lineage-level perspective complements the view from population, individual or gene-level analyses. Considering the distribution of environmental tolerance on phylogenies allows us to ask how frequently tolerance of stress evolves, and whether it has any apparent influence on macroevolutionary dynamics of trait gain and loss and speciation and extinction rates. We can also explore general patterns of cross tolerance by testing whether some traits enhance or decrease the chance of evolving particular stress tolerances, over and above the degree of co-occurrence of traits expected in related taxa. We can test our macroevolutionary hypotheses by comparing the observed distribution of tolerant taxa on phylogenies to those predicted under alternative models, and we can use simulations based on candidate macroevolutionary models to examine whether they provide a plausible explanation of the observed patterns of stress-tolerant taxa. We can combine the strengths of macroevolutionary analyses, using phylogenetic information on relatedness and evolutionary history, with the strengths of macroecological analysis, using distribution data to infer environmental stress tolerance limits and dispersal potential, to better predict both current stress tolerance of species and, potentially, their future adaptability under shifting environmental extremes.

The example of salt tolerance in angiosperms illustrates some of the interesting patterns that can be revealed by this approach. Counter to expectation, salt tolerance appears to be surprisingly easy to evolve with many hundreds of origins within diverse angiosperm lineages. But it does appear to be intriguingly difficult to maintain, with frequent loss of salt tolerance or extinction of halophyte lineages, so that many identified halophyte lineages are young in evolutionary terms and have few, if any, salt-tolerant relatives. Some lineages seem more likely to evolve salt tolerance than others, potentially due to cross-tolerance mechanisms or "enabling traits". Model comparison suggests that salt tolerance can be a "macroevolutionary self-destructive" trait, gained often but frequently lost by reversal or extinction. Salt tolerance can be predicted from species distribution data, but this prediction is more accurate when it explicitly incorporates both macroevolutionary and macroecological models of evolutionary dynamics of environmental stress tolerance.

ACKNOWLEDGMENTS

This research was funded by Australian Research Council (DP160103915).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Lindell Bromham https://orcid.org/0000-0003-2202-2609

REFERENCES

Ackerly, D. D., Schwilk, D. W., & Webb, C. O. (2006). Niche evolution and adaptive radiation: Testing the order of trait divergence. *Ecology*, *87*(sp7), S50–S61. https://doi.org/10.1890/0012-9658(2006)87[50:Neaart]2.0.Co;2

Agnarsson, I., Avilés, L., Coddington, J. A., & Maddison, W. P. (2006). Sociality in theridiid spiders: Repeated origins of an evolutionary dead end. *Evolution*, *60*, 2342–2351.

Anacker, B. L., & Strauss, S. Y. (2014). The geography and ecology of plant speciation: Range overlap and niche divergence in sister species. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1778), 20132980.

Anderson, T. M., Shaw, J., & Olff, H. (2011). Ecology's cruel dilemma, phylogenetic trait evolution and the assembly of Serengeti plant communities. *Journal of Ecology*, *99*(3), 797–806.

Araújo, M. B., & Pearson, R. G. (2005). Equilibrium of species' distributions with climate. *Ecography*, *28*, 693–695.

Aronson, J. A. (1989). *HALOPH: A data base of salt tolerant plants of the world*. Tucson, AZ: Office of Arid Land Studies, University of Arizona.

Bennett, T., Flowers, T., & Bromham, L. (2013). Repeated evolution of salt-tolerance in grasses. *Biology Letters*, *9*(2), 20130029.

Bijlsma, R., & Loeschcke, V. (2005). Environmental stress, adaptation and evolution: An overview. *Journal of Evolutionary Biology*, *18*(4), 744–749. https://doi.org/10.1111/j.1420-9101.2005.00962.x

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. *Biological Journal of the Linnean Society*, *162*(4), 543–557.

Bowler, C., & Fluhr, R. (2000). The role of calcium and activated oxygens as signals for controlling cross-tolerance. *Trends in Plant Science*, *5*(6), 241–246.

Bromham, L. (2016). Testing hypotheses in macroevolution. *Studies in History and Philosophy of Science Part A*, *55*, 47–59.

Bromham, L., & Bennett, T. (2014). Salt tolerance evolves more frequently in C4 grass lineages. *Journal of Evolutionary Biology*, *27*(3), 653–659.

Bromham, L., Hua, X., & Cardillo, M. (2016). Detecting macroevolutionary self-destruction from phylogenies. *Systematic Biology*, *65*(1), 109–127.

Bui, E. N., Thornhill, A., & Miller, J. T. (2014). Salt-and alkaline-tolerance are linked in acacia. *Biology Letters*, *10*(7), 20140278.

Capiati, D. A., País, S. M., & Téllez-Iñón, M. T. (2006). Wounding increases salt tolerance in tomato plants: Evidence on the participation of calmodulin-like activities in cross-tolerance signalling. *Journal of Experimental Botany*, *57*(10), 2391–2400.

Cheeseman, J. M. (1988). Mechanisms of salinity tolerance in plants. *Plant Physiology*, *87*(3), 547–550.

Christin, P.-A., Osborne, C. P., Chatelet, D. S., Columbus, J. T., Besnard, G., Hodkinson, T. R., … Edwards, E. J. (2013). Anatomical enablers and the evolution of C4 photosynthesis in grasses. *Proceedings of the National Academy of Sciences*, *110*(4), 1381–1386.

Cowling, R. M., Rundel, P. W., Lamont, B. B., Arroyo, M. K., & Arianoutsou, M. (1996). Plant diversity in Mediterranean-climate regions. *Trends in Ecology & Evolution*, *11*(9), 362–366.

Crisp, M. D., Burrows, G. E., Cook, L. G., Thornhill, A. H., & Bowman, D. M. J. S. (2011). Flammable biomes dominated by eucalypts originated at the cretaceous–Palaeogene boundary. *Nature Communications*, *2*(1), 193. https://doi.org/10.1038/ncomms1191

Davis, M. P., Midford, P. E., & Maddison, W. (2013). Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. *BMC Evolutionary Biology*, *13*(1), 38. https://doi.org/10.1186/1471-2148-13-38

Day, E., Hua, X., & Bromham, L. (2016). Is specialization an evolutionary dead end? Testing for differences in speciation, extinction and trait transition rates across diverse phylogenies of specialists and generalists. *Journal of Evolutionary Biology*, *29*(6), 1257–1267.

Deinlein, U., Stephan, A. B., Horie, T., Luo, W., Xu, G., & Schroeder, J. I. (2014). Plant salt-tolerance mechanisms. *Trends in Plant Science*, *19*(6), 371–379. https://doi.org/10.1016/j.tplants.2014.02.001

Doubnerová, V., & Ryšlavá, H. (2011). What can enzymes of C4 photosynthesis do for C3 plants under stress? *Plant Science*, *180*(4), 575–583.

Duchêne, D. A., Hua, X., & Bromham, L. (2017). Phylogenetic estimates of diversification rate are affected by molecular rate variation. *Journal of Evolutionary Biology*, *30*(10), 1884–1897.

Eallonardo, A. S., Leopold, D. J., Fridley, J. D., & Stella, J. C. (2013). Salinity tolerance and the decoupling of resource axis plant traits. *Journal of Vegetation Science*, *24*(2), 365–374. https://doi.org/10.1111/j.1654-1103.2012.01470.x

Edwards, E. J., & Smith, S. A. (2010). Phylogenetic analyses reveal the shady history of C4 grasses. *Proceedings of the National Academy of Sciences*, *107*(6), 2532–2537.

Ferrier, S., Manion, G., Elith, J., & Richardson, K. (2007). Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. *Diversity and Distributions*, *13*(3), 252–264.

FitzJohn, R. G., Maddison, W. P., & Otto, S. P. (2009). Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. *Systematic Biology*, *58*(6), 595–611.

Flowers, T., & Flowers, S. (2005). Why does salinity pose such a difficult problem for plant breeders? *Agricultural Water Management*, *78*(1–2), 15–24.

Flowers, T. J., & Colmer, T. D. (2008). Salinity tolerance in halophytes. *The New Phytologist*, *179*, 945–963.

Flowers, T. J., & Colmer, T. D. (2015). Plant salt tolerance: Adaptations in halophytes. *Annals of Botany*, *115*(3), 327–331.

Flowers, T. J., Galal, H. K., & Bromham, L. (2010). Evolution of halophytes: Multiple origins of salt tolerance in land plants. *Functional Plant Biology*, *37*(7), 604–612.

Gamisch, A. (2016). Notes on the statistical Power of the binary state speciation and extinction (BiSSE) model. *Evolutionary Bioinformatics*, *12*, EBO.S39732. https://doi.org/10.4137/EBO.S39732

Gamisch, A., Fischer, G. A., & Comes, H. P. (2015). Multiple independent origins of auto-pollination in tropical orchids (Bulbophyllum) in light of the hypothesis of selfing as an evolutionary dead end. *BMC Evolutionary Biology*, *15*(1), 192. https://doi.org/10.1186/s12862-015-0471-5

Goldberg, E. E., Kohn, J. R., Lande, R., Robertson, K. A., Smith, S. A., & Igić, B. (2010). Species selection maintains self-incompatibility. *Science*, *330*(6003), 493–495.

Graham, C. H., & Fine, P. V. (2008). Phylogenetic beta diversity: Linking ecological and evolutionary processes across space in time. *Ecology Letters*, *11*(12), 1265–1277.

Haak, D. C., Ballenger, B. A., & Moyle, L. C. (2014). No evidence for phylogenetic constraint on natural defense evolution among wild tomatoes. *Ecology*, *95*(6), 1633–1641.

Heath, T. A., Zwickl, D. J., Kim, J., & Hillis, D. M. (2008). Taxon sampling affects inferences of macroevolutionary processes from phylogenetic trees. *Systematic Biology*, *57*(1), 160–166.

Hinchliff, C. E., Smith, S. A., Allman, J. F., Burleigh, J. G., Chaudhary, R., Coghill, L. M., … Cranston, K. A. (2015). Synthesis of phylogeny and taxonomy into a comprehensive tree of life. *Proceedings of the National Academy of Sciences*, *112*(41), 12764–12769. https://doi.org/10.1073/pnas.1423041112

Hopper, S. D., & Gioia, P. (2004). The southwest Australian floristic region: Evolution and conservation of a global hot spot of biodiversity. *Annual Review of Ecology and Systematics*, *35*, 623–650.

Hua, X., & Bromham, L. (2015). Phylometrics: An R package for detecting macroevolutionary patterns, estimating errors on phylogenetic metrics, and backward tree simulation. *Methods in Ecology and Evolution*, *7*(7), 806–810.

Hua, X., Cardillo, M., and Bromham, L. (2019). Tracking niche change through time: simultaneous inference of environmental niche evolution and estimation of contemporary niches. bioRxiv, 2019.2012.2029.890608. https://doi.org/10.1101/2019.12.29.890608.

Hua, X., & Lanfear, R. (2018). The influence of non-random species sampling on macroevolutionary and macroecological inference from phylogenies. *Methods in Ecology and Evolution*, *9*(5), 1353–1362.

Johnson, M. T., Ives, A. R., Ahern, J., & Salminen, J. P. (2014). Macroevolution of plant defenses against herbivores in the evening primroses. *New Phytologist*, *203*(1), 267–279.

Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., … Wirth, C. (2020). TRY plant trait database–Enhanced coverage and open access. *Global Change Biology*, *26*(1), 119–188. https://doi.org/10.1111/gcb.14904

Leprieur, F., Albouy, C., De Bortoli, J., Cowman, P. F., Bellwood, D. R., & Mouillot, D. (2012). Quantifying phylogenetic beta diversity: Distinguishing between 'true' turnover of lineages and phylogenetic diversity gradients. *PLoS One*, *7*(8), e42760.

Liu, H., Edwards, E. J., Freckleton, R. P., & Osborne, C. P. (2012). Phylogenetic niche conservatism in C 4 grasses. *Oecologia*, *170*(3), 835–845.

Maddison, W. P., Midford, P. E., & Otto, S. P. (2007). Estimating a binary Character's effect on speciation and extinction. *Systematic Biology*, *56*(5), 701–710. https://doi.org/10.1080/10635150701607033

Maynard Smith, J. (1978). *The evolution of sex*, Cambridge: Cambridge University Press.

Miller, J. A., & Holloway, P. (2015). Incorporating movement in species distribution models. *Progress in Physical Geography*, *39*, 837–849.

Moray, C., Goolsby, E. W., & Bromham, L. (2016). The phylogenetic association between salt tolerance and heavy metal hyperaccumulation in angiosperms. *Evolutionary Biology*, *43*(1), 119–130.

Moray, C., Hua, X., & Bromham, L. (2015). Salt tolerance is evolutionarily labile in a diverse set of angiosperm families. *BMC Evolutionary Biology*, *15*(1), 90.

Mudgal, V., Madaan, N., & Mudgal, A. (2010). Biochemical mechanisms of salt tolerance in plants: A review. *International Journal of Botany*, *6*(2), 136–143.

Munns, R. (2005). Genes and salt tolerance: Bringing them together. *New Phytologist*, *167*(3), 645–663. https://doi.org/10.1111/j.1469-8137.2005.01487.x

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, *59*, 651–681.

Ng, J., & Smith, S. D. (2018). Why are red flowers so rare? Testing the macroevolutionary causes of tippiness. *Journal of Evolutionary Biology*, *31*(12), 1863–1875.

Osborne, C. P., & Sack, L. (2012). Evolution of C4 plants: A new hypothesis for an interaction of CO2 and water relations mediated by plant hydraulics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1588), 583–600.

Pearse, I. S., Aguilar, J., Schroder, J., & Strauss, S. Y. (2017). Macroevolutionary constraints to tolerance: Trade-offs with drought tolerance and phenology, but not resistance. *Ecology*, *98*(11), 2758–2772. https://doi.org/10.1002/ecy.1995

Peterson, A. T., Soberón, J., Pearson, R. G., Anderson, R. P., Martínez-Meyer, E., Nakamura, M., & Araújo, M. B. (2011). *Ecological niches and geographic distributions (MPB-49)*, Princeton: Princeton University Press.

Puniran-Hartley, N., Hartley, J., Shabala, L., & Shabala, S. (2014). Salinity-induced accumulation of organic osmolytes in barley and wheat leaves correlates with increased oxidative stress tolerance: In planta evidence for cross-tolerance. *Plant Physiology and Biochemistry*, *83*, 32–39.

Rajakaruna, N. (2018). Lessons on evolution from the study of edaphic specialization. *The Botanical Review*, *84*(1), 39–78.

Rozema, J., & Schat, H. (2013). Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. *Environmental and Experimental Botany*, *92*, 83–95.

Santos, J., Al-Azzawi, M., Aronson, J., & Flowers, T. J. (2016). eHALOPH a database of salt-tolerant plants: Helping put halophytes to work. *Plant and Cell Physiology*, *57*(1), e10.

Saslis-Lagoudakis, C. H., Hua, X., Bui, E., Moray, C., & Bromham, L. (2015). Predicting species' tolerance to salinity and alkalinity using distribution data and geochemical modelling: A case study using Australian grasses. *Annals of Botany*, *115*, 343–351. https://doi.org/10.1093/aob/mcu248

Schwander, T., & Crespi, B. J. (2008). Twigs on the tree of life? Neutral and selective models for integrating macroevolutionary patterns with microevolutionary processes in the analysis of asexuality. *Molecular Ecology*, *18*(1), 28–42.

Serrano-Serrano, M. L., Rolland, J., Clark, J. L., Salamin, N., & Perret, M. (2017). Hummingbird pollination and the diversification of angiosperms: An old and successful association in Gesneriaceae. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1852), 20162816.

Shabala, S. (2013). Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops. *Annals of Botany*, *112*, 1209–1221.

Shahbaz, M., & Ashraf, M. (2013). Improving salinity tolerance in cereals. *Critical Reviews in Plant Sciences*, *32*, 237–249.

Simon, M. F., Grether, R., de Queiroz, L. P., Skema, C., Pennington, R. T., & Hughes, C. E. (2009). Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences*, *106*(48), 20359–20364.

Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., & Savouré, A. (2015). Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Annals of Botany*, *115*(3), 433–447.

Smith, S. A., & Brown, J. W. (2018). Constructing a broadly inclusive seed plant phylogeny. *American Journal of Botany*, *105*(3), 302–314. https://doi.org/10.1002/ajb2.1019

Stadler, T. (2012). How can we improve accuracy of macroevolutionary rate estimates? *Systematic Biology*, *62*(2), 321–329. https://doi.org/10.1093/sysbio/sys073

Swenson, N. G. (2011). Phylogenetic beta diversity metrics, trait evolution and inferring the functional beta diversity of communities. *PLoS One*, *6*(6), e21264. https://doi.org/10.1371/journal.pone.0021264

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

Title, P. O., & Rabosky, D. L. (2017). Do macrophylogenies yield stable macroevolutionary inferences? *An Example from Squamate Reptiles. Systematic Biology*, *66*(5), 843–856. https://doi.org/10.1093/sysbio/syw102

Tripp, E. A., & Manos, P. S. (2008). Is floral specialization an evolutionary dead-end? Pollination system transitions in Ruellia (Acanthaceae). *Evolution*, *62*(7), 1712–1737.

Verdu, M., & Pausas, J. G. (2007). Fire drives phylogenetic clustering in Mediterranean Basin woody plant communities. *Journal of Ecology*, *95*, 1316–1323.

Webb, C. O., Ackerly, D. D., McPeek, M. A., & Donoghue, M. J. (2002). Phylogenies and community ecology. *Annual Review of Ecology and Systematics*, *33*, 475–505.

Webb, C. O., & Donoghue, M. J. (2005). Phylomatic: Tree assembly for applied phylogenetics. *Molecular Ecology Notes*, *5*(1), 181–183.

Williams, G. C. (1975). *Sex and evolution*, Princeton: Princeton University Press.

Zurell, D. (2017). Integrating demography, dispersal and interspecific interactions into bird distribution models. *Journal of Avian Biology*, *48*, 1505–1516.

How to cite this article: Bromham L, Hua X, Cardillo M. Macroevolutionary and macroecological approaches to understanding the evolution of stress tolerance in plants. *Plant Cell Environ*. 2020;43:2832–2846. https://doi.org/10.1111/pce.13857