Evolutionary origins of abnormally large shoot sodium accumulation in non-saline environments within the Caryophyllales

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Summary

- The prevalence of sodium (Na) "hyperaccumulator" species, which exhibit abnormally large shoot sodium concentrations ($[Na]_{shoot}$) when grown in non-saline environments, was investigated among angiosperms in general and within the Caryophyllales order in particular.
- Shoot Na concentrations were determined in 334 angiosperm species, representing 35 orders, grown hydroponically in a non-saline solution.
- Many Caryophyllales species exhibited abnormally large $[Na]_{shoot}$ when grown hydroponically in a non-saline solution. The bimodal distribution of the log-normal $[Na]_{shoot}$ of species within the Caryophyllales suggested at least two distinct $[Na]_{shoot}$ phenotypes within this order. Mapping the trait of Na-hyperaccumulation onto the phylogenetic relationships between Caryophyllales families, and between subfamilies within the Amaranthaceae, suggested that the trait evolved several times within this order: in an ancestor of the Aizoaceae, but not the Phytolaccaceae or Nyctaginaceae, in ancestors of several lineages formerly classified as Chenopodiaceae, but not in the Amaranthaceae *sensu stricto*, and in ancestors of species within the Cactaceae, Portulacaceae, Plumbaginaceae, Tamaricaceae and Polygonaceae.
- In conclusion, a disproportionate number of Caryophyllales species behave as Na-hyperaccumulators and multiple evolutionary origins of this trait can be identified within this order.

Key words: Aizoaceae, Amaranthaceae, Caryophyllales, halophyte, hyperaccumulation, phylogeny, shoot, sodium (Na).

Introduction

Sodium (Na) is not considered to be an essential element for plants (White & Brown 2010) although it is required (in micronutrient quantities) for the C_4 photosynthetic pathway (Cheeseman, 2015) and some halophytes (euhalophytes) grow better when supplied Na (Greenway & Munns, 1980; Albert, 1982; Flowers & Colmer, 2008; Munns & Tester, 2008; Rozema & Schat, 2013). In addition, in some environments, for example where there is low K^+ phytoavailability, plant growth can benefit from a source of Na since Na^+ can replace K^+ as a cationic osmoticum in the vacuole (White, 2013). The accumulation of excessive Na concentrations in plant tissues is, however, detrimental to plant growth since Na^+ interferes with metabolism in the cytoplasm, mitochondria and plastids (Flowers *et al.*, 2015).

It is estimated that >6% of the world's land, and 5-15% of the world's agricultural land, is adversely affected by its Na concentration through either salinity or sodicity (Munns & Tester, 2008). Saline soils are generally dominated by NaCl, although there are often significant concentrations of Ca^{2+}, Mg^{2+}, SO_4^{2-} and CO_3^{2-}. They are defined as having <15% of their exchangeable cations as Na^+ and soil solutions with electrical conductivity (EC_e) >2 dS m^{-1} in a saturated paste extract, which equates to a NaCl concentration of 20 mM, and pH <8.5. Sodic (alkali) soils are generally dominated by Na_2CO_3 and are defined as having >15% of their exchangeable cations as Na^+ and soil solutions with EC_e >2 dS m^{-1} and pH >8.5 in a saturated paste extract. Saline-sodic soils have >15% of their exchangeable cations as Na^+ and soil solutions with EC_e >2 dS m^{-1} and pH <8.5 in a saturated paste extract.

Halophytes are generally defined as plants that inhabit saline environments or that complete their life cycles in the presence of large concentrations of ions (≥ 200 mM), most commonly NaCl, in the root-zone (Flowers & Colmer, 2008). They can be further classified into miohalophytes, which exhibit maximal growth in non-saline environments, and euhalophytes, which exhibit maximal growth under saline conditions (Greenway & Munns, 1980). Halophytes tolerating EC_e >8.0 dS m^{-1} measured in a saturated paste extract (approximately 80 mM NaCl) comprise <0.5% of angiosperm species (1,490/352,000 species), but are present in at least 33 orders and 110-120 families of flowering plants (The Plant List, 2013; Flowers *et al.*, 2016). It has been suggested that halophytism is an evolutionarily-labile character that has arisen independently in many angiosperm lineages from pre-adapted genotypes (Flowers *et al.*, 2010; Kadereit *et al.*, 2012; Saslis-Lagoudakis *et*

al., 2014; Bromham, 2015; Cheeseman, 2015). Families with a large proportion of halophytes (>10% of species in a family) occur in the Alismatales, Brassicales, Caryophyllales, Ericales, Fabales, Malphigiales, Piperales, Poales, Sapindales and Saxifragales (Saslis-Lagoudakis *et al.*, 2014; Flowers *et al.*, 2016).

Halophytes can also be grouped into "ionotypes", which are defined as characteristic ionomic features of plant species that are conserved in diverse environments (Albert & Popp, 1977; Gorham *et al.*, 1980; Albert *et al.*, 2000; Flowers & Colmer, 2008; White *et al.*, 2012). Commelinid monocots (e.g. Poaceae, Cyperaceae, Juncaceae) are classed as "Na-excluders" and generally exhibit lower shoot Na concentrations ($[Na]_{shoot}$) than other angiosperms growing in the same environment and Na/K quotients less than unity, whilst many eudicots are characterised by comparatively large $[Na]_{shoot}$ and tissue Na/K quotients greater than unity (Albert & Popp, 1977; Gorham *et al.*, 1980; Albert, 1982; Flowers & Colmer, 2008; Yang *et al.*, 2012). Several families in the Caryophyllales (Amaranthaceae [Chenopodioideae], Caryophyllaceae, Tamaricaceae) exhibit exceptionally large $[Na]_{shoot}$ and tissue Na/K quotients when grown in saline environments (Albert & Popp, 1977; Gorham *et al.*, 1980; Albert, 1982; Flowers & Colmer, 2008; Yang *et al.*, 2012; Zhang *et al.*, 2012). It has also been observed that some Caryophyllales species have exceptionally large $[Na]_{shoot}$ even when grown in non-saline environments (Collander, 1941; Patel *et al.*, 1980; Glenn & O'Leary, 1984; Broadley *et al.*, 2004). For example, in a phylogenetically-balanced study of the ionomes of 117 angiosperm species belonging to 25 orders grown hydroponically in a non-saline solution containing 0.1 mM Na, it was noted that $[Na]_{shoot}$ varied significantly among eudicot orders ($P<0.05$) and that three of the seven Caryophyllales species studied had conspicuously large $[Na]_{shoot}$ (Broadley *et al.*, 2004). It has been suggested that Na might have a special role in the biology of euhalophyte Caryophyllales, whose maximal growth requires Na accumulation (Flowers & Colmer, 2008), and that the characteristic ionome of the Caryophyllales might reflect their unusual ecology (White *et al.*, 2015). Although Caryphyllales species can inhabit a variety of biomes worldwide, they comprise a significant proportion of the flora of many deserts (Fahn & Cutler, 1992), coastal regions (Kadereit *et al.*, 2012), and soils with unbalanced mineral composition for plant nutrition, such as gypseous (Moore *et al.*, 2014) and ultramafic/serpentine (White & Pongrac, 2016) soils.

The Caryophyllales order comprises over 11,000 species currently partitioned into about 700 genera and 38 families (The Plant List, 2013; APGIV, 2016). About 5% of species in

the Caryophyllales are halophytes and the order contains 35-40% of all known halophytic angiosperm species (Flowers *et al.*, 2010, 2016; Saslis-Lagoudakis *et al.*, 2014). Of the most populous families in the Caryophyllales (>50 species) the halophytic character is particularly prevalent in the Amaranthaceae (17.3% species), Frankeniaceae (16.7% species) and Tamaricaceae (31.1% species). In contrast to observations on other angiosperm orders, the halophytic character appears to be rarely lost in Caryophyllales lineages, such as the Chenopodioideae and Tamaricaceae, once it has evolved (Bromham, 2015). It has been suggested that the halophytic character might evolve from ancestors with a general complement of stress-tolerance traits that enable lineages to adapt to a wide range of environmental challenges (Kadereit *et al.*, 2012; Saslis-Lagoudakis *et al.*, 2014; Bromham, 2015). It is, therefore, noteworthy that the Caryophyllales order contains many succulent species (Kadereit *et al.*, 2012; Rozema & Schat, 2013), many species that possess salt glands, which are specialised multicellular structures that excrete salt onto the leaf surface, or bladder cells, which are modified trichomes that accumulate salt and then burst (Thomson *et al.*, 1988; Fahn & Cutler, 1992; Salama *et al.*, 1999; Flowers *et al.*, 2010; LoPresti, 2014), many species exhibiting C_4 and CAM photosynthetic pathways (Silvera *et al.*, 2010; Sage *et al.,* 2011; Kadereit *et al.*, 2012), many species that hyperaccumulate potentially toxic elements (White & Pongrac, 2016), and many species adapted to arid (Ehleringer *et al.*, 1997) or alkaline (Yang *et al.*, 2012) environments. The C_4 photosynthetic pathway has evolved many times within the Caryophyllales (Sage *et al.*, 2011) and Kadereit *et al.* (2012) observed that the rate of gain of the C_4 photosynthetic character was greater in salt tolerant Chenopodioideae lineages, which they attributed to shared adaptations between C_4 photosynthesis and salt tolerance as part of a wider drought tolerance syndrome. A similar dependency of the evolution of C_4 photosynthesis with succulence and coastal habitat was also observed (Kadereit *et al.*, 2012). Crassulacean Acid Metabolism has also evolved many times within the Caryophyllales and is associated with succulence and other traits enabling water use efficiency in arid or saline environments (Edwards & Ogburn, 2012).

The present study investigated the prevalence of "Na-hyperaccumulator" species, which exhibit abnormally large $[Na]_{shoot}$ (>4 mg Na g^{-1} dry matter) when grown in non-saline conditions (<20 mM Na^+ in the rhizosphere solution), among the angiosperms in general and the Caryophyllales in particular. The prevalence of this phenomenon among angiosperms is currently unknown and this study provides an original insight to its occurrence and

153 evolutionary origins within the Caryophyllales order. It is observed that only the
154 Caryophyllales species *Atriplex hortensis* L. and *Beta vulgaris* L. of the ten halophytic species
155 studied, representing eight angiosperm orders, behaved as Na-hyperaccumulators when
156 grown in compost. Similarly, when 334 angiosperm species representing 35 angiosperm
157 orders were grown hydroponically in a non-saline solution containing 0.1 mM Na a
158 disproportionate number of Caryophyllales species exhibited abnormally large $[Na]_{shoot}$. The
159 bimodal distribution of the log-normal $[Na]_{shoot}$ of species within the Caryophyllales
160 suggested at least two distinct $[Na]_{shoot}$ phenotypes within this order. Mapping the trait of
161 Na-hyperaccumulation in non-saline environments onto the phylogenetic relationships
162 between Caryophyllales families (Crawley & Hilu, 2012; Hernández-Ledesma *et al.*, 2015;
163 Yang *et al.*, 2015), and between subfamilies within the Amaranthaceae, suggested that the
164 trait had evolved several times within this order: in an ancestor of the Aizoaceae, but not
165 the Phytolaccaceae or Nyctaginaceae, in ancestors of several lineages formerly classified as
166 Chenopodiaceae, but not in the Amaranthaceae *sensu stricto*, and possibly in ancestors of
167 species within the Cactaceae, Portulacaceae, Plumbaginaceae, Tamaricaceae and
168 Polygonaceae. It is possible that the ability to hyperaccumulate Na^+ might benefit plants by
169 providing an alternative osmoticum to K^+, especially in environments with low K availability
170 (White, 2013). Thus, Na-hyperaccumulation might have served Caryophyllales during their
171 evolution in overcoming the selection pressures associated with the colonisation of arid or
172 saline environments, which require succulence and water conservation (Fahn & Cutler,
173 1992; Nobel, 2003; Flowers & Colmer, 2008; Kadereit *et al.*, 2012).
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176 **Materials and Methods**
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178 Responses of halophytic species from different angiosperm orders to salinity
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180 Responses to salinity were studied in ten halophytic species, from eight angiosperm orders,
181 catalogued in the eHALOPH Halophytes Database (Flowers *et al.*, 2016). These comprised:
182 *Ammi visnaga* (L.) Lam. (Apiaceae, Apiales), *Asparagus officinalis* L. (Asparagaceae,
183 Asparagales), *Atriplex hortensis* L. (Amaranthaceae, Caryophyllales), *Beta vulgaris* L.
184 (Amaranthaceae, Caryophyllales), *Casuarina cunninghamiana* Miq. (Casuarinaceae, Fagales),

185 *Colubrina asiatica* (L.) Brongn. (Rhamnaceae, Rosales), *Hibiscus tilliaceus* L. (Malvaceae,
186 Malvales), *Hordeum jubatum* L. (Poaceae, Poales), *Kosteletzkya virginica* (L.) C. Presl ex A.
187 Gray (Malvaceae, Malvales), *Lobularia maritima* (L.) Desv. (Brassicaceae, Brassicales),
188 *Plantago maritima* L. (Plantaginaceae, Lamiales) and *Scaevola crassifolia* Labill.
189 (Goodeniaceae, Asterales). Species were chosen on the basis of their availability from
190 suppliers and their ability to grow in the glasshouse. Seeds of all species were obtained from
191 Chiltern Seeds (Wallingford, UK) except *C. cunninghamiana*, *H. tilliaceous*, *K. virginica* and *P.*
192 *maritima*, which were obtained from Rareexoticseeds (Montreal, Canada), Kenni Koala's
193 Aussie Seed Store (Australia), Floridawildflowers (Crescent City, Florida, USA) and Scotia
194 Seeds (Brechin, UK), respectively. Seeds were germinated in the dark at between 10 °C and
195 25 °C, according to species requirements, on the surface of filter paper moistened with
196 deionised water. Once a radicle was observed, individual seedlings were transplanted to
197 rockwool plugs (2.5 cm by 2.5 cm by 4 cm; Grodan, Hedehusene, Denmark) held in plastic
198 trays in a glasshouse compartment at The James Hutton Institute, Dundee (UK; latitude
199 56°27'26'' N, longitude 3°4'17'' W) in which the experiment was subsequently performed
200 and irrigated with tap water containing 0.14 mM Na. The glasshouse compartment
201 maintained a maximum of 25 °C by day and a minimum of 15 °C at night using automatic
202 venting and supplementary heating.

203 Established seedlings were transferred to pots containing 1 L Levington Professional
204 compost (ICL, Ipswich, UK) prior to the experiment. Two sets of plants, with up to 12
205 replicate plants per species in each set, were exposed to either non-saline or saline
206 irrigation. Plants were irrigated with 100 mL solution per week. Plants receiving the non-
207 saline treatment were irrigated with tap water containing 0.14 mM Na. The experiment was
208 initiated by increasing the NaCl concentration in the irrigation water of the saline treatment
209 to 50 mM for the first week, then 150 mM NaCl for the second week and finally 300 mM for
210 the third week. Plants were harvested on 12th December 2014, three weeks after the first
211 addition of NaCl to the saline irrigation water. The fresh weight (FW) of whole shoots was
212 determined immediately, then samples were dried in an oven at 70 °C to a constant weight
213 and their dry matter (DM) determined. Dried samples were milled to a powder using a ball
214 mill (C + N Laboratory Mill; Christy and Norris Ltd., Chelmsford, UK), digested using HNO_3 in
215 sealed tubes in a microwave oven (MARS Xpress, CEM Corporation, Matthews, NC, USA),
216 cleared using H_2O_2, and analysed for sodium (Na) concentration using inductively coupled

plasma-mass spectrometry (ICP-MS; ELAN DRCe, PerkinElmer, Waltham, MA, USA) as described by White *et al.* (2012).

Phylogenetic effects on shoot sodium concentrations in plants grown hydroponically in a non-saline solution

Phylogenetic effects on shoot Na concentrations in angiosperm species were assessed by combining data from six glasshouse experiments in which plants were grown hydroponically using a Nutrient Film Technique (NFT) essentially as described by Broadley *et al.* (2003). The final dataset comprised 334 species from 35 orders (Table S1). In all experiments, seeds were germinated in the dark on the surface of filter paper moistened with deionised water at temperatures between 4 °C and 25 °C depending on their requirements. Once a radicle was observed, individual seedlings were transplanted to rockwool plugs (2.5 cm by 2.5 cm by 4 cm; Grodan, Hedehusene, Denmark) held in plastic trays and irrigated with tap water. Plastic trays were either placed in a weaning room at 25 °C or in the glasshouse compartment in which experiments were subsequently performed. Once seedlings were established, the rockwool plugs containing plants were transferred to the NFT system. Whenever possible, two rockwool plugs constituted each replicate and up to six replicates were obtained for each plant species. For experiments at both Warwick-HRI, Wellesbourne (UK; latitude 52°12’18’’ N, longitude 1°36’00’’ W) and The James Hutton Institute, the glasshouse maintained a maximum of 20 °C by day and a minimum of 15 °C at night using automatic venting and supplementary heating. The recirculating nutrient solution contained 2 mM $Ca(NO_3)_2$, 2 mM NH_4NO_3, 0.75 mM $MgSO_4$, 0.5 mM KOH, 0.25 mM KH_2PO_4, 0.1 mM FeNaEDTA, 30 µM H_3BO_3, 25 µM $CaCl_2$, 10 µM $MnSO_4$, 3 µM $CuSO_4$, 1 µM $ZnSO_4$ and 0.5 µM Na_2MoO_4. This was adjusted daily to pH 6, with H_2SO_4, and solutions were replaced completely once or twice each week. Seedlings were harvested during the exponential growth phase, 18-73 days after transfer to the hydroponic system depending upon plant growth rate. Whenever possible, shoots were separated into leaves and stems. The FW of whole shoots or leaves was determined immediately then samples were dried in an oven at 70 - 80 °C to a constant weight and their DM determined. Dried samples were milled to a powder using a ball mill, acid digested, and their Na concentrations determined either by inductively coupled plasma emission spectrometry (JY24; Jobin-Yvon, Longjumeau, France)

as described by Broadley *et al.* (2003; Experiments 1-4) or by ICP-MS as described by White *et al.* (2012; Experiments 5 and 6).

Experiment 1, described by Broadley *et al.* (2004), was undertaken in a glasshouse compartment at Warwick-HRI between July and October 2001 to survey calcium (Ca), potassium (K), magnesium (Mg), Na, organic-N and phosphorus (P) concentrations in leaves of a phylogenetically-balanced set of 117 angiosperm species belonging to 25 orders. Experiments 2A, 2B and 2C were undertaken sequentially in a glasshouse compartment at Warwick-HRI between May and November 2003 to survey Ca concentrations in leaves of Magnoliid and monocot orders, with replication at the taxonomic level of the family. Six species representing three Magnoliid orders, 54 species representing eight monocot orders, and nine other angiosperm species were grown in this experiment. Experiment 3, described by White *et al.* (2007), was undertaken in a glasshouse compartment at Warwick-HRI between July and August 2004 to survey selenium (Se) concentrations in leaves of 35 angiosperm species chosen to represent the range of ecological strategies for Se accumulation reported in angiosperms. Experiment 4, described by White *et al.* (2015), was undertaken in a glasshouse compartment at Warwick-HRI between June and August 2004 to survey leaf concentrations of Ca and Mg in as many Caryophyllales families as possible, with replication at the taxonomic level of the genus. Forty-six Caryophyllales species were studied, representing eight families and 29 genera, together with 33 other angiosperm species. Experiment 5 was undertaken in a glasshouse compartment at The James Hutton Institute between July and October 2011 to survey leaf Ca and Mg concentrations in a range of serpentine and non-serpentine plant species. These included 28 Caryophyllales species and 35 other angiosperm species. Experiment 6 was undertaken in a glasshouse compartment at The James Hutton Institute between July and November 2015 to survey leaf Ca and Mg concentrations in a range of Arecaceae species, with replication at the taxonomic level of the genus. Twenty three Arecaceae species were studied, representing six genera, together with 11 other angiosperm species. Each Experiment had several species in common with other Experiments allowing cross comparisons (Table S1). In total, 53 species, representing 22 families and 15 orders, were grown in more than one Experiment.

Statistics

Data are expressed as mean and standard error or standard deviation of the mean of n observations. Statistical differences between treatments were assessed for each species by Student's t-test. Estimates of variation in $[Na]_{shoot}$ were assigned between and within orders (*n*=35), families (*n*=79) and species (*n*=334) using analyses of variance (ANOVA). All statistical analyses were performed using R 3.3.0 (R Core Team, 2016) using a linear model of: $[Na]_{shoot}$ ~ Order+Family+Species.

Results

Ten halophytic angiosperm species were grown in compost in pots that were irrigated with either non-saline or saline solution. The shoot fresh weight (FW) of most of these species did not differ significantly between plants that were irrigated with non-saline and saline solutions (Table 1). However, the shoot FWs of *Asparagus officinalis* (*P*=0.0193) and *Kosteletzkya virginica* (*P*=0.0430) were less in plants irrigated with saline solutions than in those irrigated with non-saline solutions, whereas the shoot FWs of *Atriplex hortensis* (*P*=0.0090) were greater in plants irrigated with a saline solutions than those irrigated with non-saline solutions. Previous studies have also suggested that halophytic *Atriplex* species grow best under slightly saline conditions (Black, 1960; Wallace *et al.*, 1973; Storey & Wyn Jones, 1979; Albert, 1982; Glenn & O'Leary, 1984; Redondo-Gómez *et al.*, 2007; Glenn *et al.*, 2012; Norman *et al.*, 2013).

The response of shoot Na concentration ($[Na]_{shoot}$) to irrigation with saline solution differed between the species studied, and they could be classified into "Na-excluder", "Na-responder" and "Na-accumulator" species (cf. Baker, 1981). Of the ten angiosperm species studied, four species appeared to exclude Na from their shoot tissues and had small $[Na]_{shoot}$ when irrigated with either non-saline and saline solutions (Table 1). These "Na-excluder" species were the two monocot species studied, *Hordeum jubatum* (Poales) and *Asparagus officinalis* (Asparagales), *Hibiscus tilliaceus* (Malvales) and *Casuarina cunninghamiana* (Fagales). Five species had relatively small $[Na]_{shoot}$ when irrigated with non-saline solution but when irrigated with saline solution their $[Na]_{shoot}$ increased to more than 10 mg g^{-1} DM. These "Na-responder" species were *Colubrina asiatica* (Rosales), *Kosteletzkya virginica* (Malvales), *Ammi visnaga* (Apiales), *Lobularia maritima* (Brassicales), *Scaevola crassifolia*

(Asterales) and *Plantago maritima* (Lamiales). The two Caryophyllales species studied, *Beta vulgaris* and *Atriplex hortensis*, both had exceptionally large $[Na]_{shoot}$ when irrigated with non-saline and saline solutions. These species could be designated "Na-accumulator" species.

The constitutively large $[Na]_{shoot}$ of "Na-accumulator" species could best be distinguished when plants were irrigated with non-saline solutions (Table 1). The distribution of this trait among angiosperms was, therefore, assessed by growing species hydroponically in a solution containing little Na as described by Broadley *et al.* (2003). Data were combined from six individual glasshouse experiments (Table S1). Since little of the variation in $[Na]_{shoot}$ (3.4%) could be attributed to environment (i.e. experiment), the $[Na]_{shoot}$ for each species was calculated as the arithmetic mean of all experiments in which the species was grown (Table S1). The proportions of the variation in $[Na]_{shoot}$ accounted for at the levels of order, family and species were 13.8%, 54.3% and 28.5%, respectively. This suggests that different plant families show distinct $[Na]_{shoot}$ concentrations. Families with the largest mean $[Na]_{shoot}$ of their constituent species were the Aizoaceae (24.47 ± 5.07 mg g^{-1} DM, *n*=7 species), Cactaceae (17.60, *n*=1 species), Melastomataceae (5.23, *n*=1 species), Portulacaceae (5.20 ± 4.60, *n*=2 species), and Ericaceae (4.51 ± 3.89, *n*=2 species). Three of these families are in the Caryophyllales order.

The $[Na]_{shoot}$ differed considerably between angiosperm species grown hydroponically in a non-saline solution (Table S1; Figure 1). Several species had mean $[Na]_{shoot}$ greater than 10 mg g^{-1} DM. These species included nine Caryophyllales species, *Beta vulgaris* (Amaranthaceae; 13.37 ± 2.35 mg g^{-1} DM, *n*=5 experiments), *Echinofossulocactus* sp. (Cactaceae; 17.60 mg g^{-1} DM, *n*=1 experiment), *Carpanthea pomeridiana* (Aizoaceae; 19.85 mg g^{-1} DM, *n*=1 experiment), *Hereroa odorata* (Aizoaceae; 20.17 mg g^{-1} DM, *n*=1 experiment), *Carpobrotus edulis* (Aizoaceae; 22.76 ± 3.31 mg g^{-1} DM, *n*=2 experiments), *Atriplex hortensis* (Amaranthaceae; 23.75 ± 1.01 mg g^{-1} DM, *n*=2 experiments), *Stigmatocarpum criniflorum* (Aizoaceae; 30.81 ± 3.98 mg g^{-1} DM, *n*=3 experiments), *Mesembryanthemum cordifolium* (Aizoaceae; 37.42 ± 6.44 mg g^{-1} DM, *n*=2 experiments) and *Dorotheanthus bellidiformis* (Aizoaceae; 40.02 mg g^{-1} DM, *n*=1 experiment), and two other angiosperm species, C*allistemon rigidus* (Myrtaceae, Myrtales; 10.31 mg g^{-1} DM, *n*=1 experiment) and *Gladiolus carneus* (Iridaceae, Asparagales; 10.50 mg g^{-1} DM, *n*=1 experiment).

The distribution of $[Na]_{shoot}$ among the angiosperm species studied did not fit a simple normal distribution (Figure 1A) and the log-normal distribution of $[Na]_{shoot}$ appeared to comprise the sum of at least three individual log-normal distributions (Figure 2A). The $[Na]_{shoot}$ of Caryophyllales species differed by several orders of magnitude, from 0.05 mg g^{-1} DM in *Lewisia cotyledon* (Montiaceae) to 40.02 mg g^{-1} DM in *Dorotheanthus bellidiformis* (Aizoaceae). The distribution of $[Na]_{shoot}$ among the Caryophyllales appeared to comprise a normal distribution (mean = 0.393, standard deviation = 0.185 mg g^{-1} DM, *n*=42 species) plus up to 19 species with abnormally large $[Na]_{shoot}$ (Figure 1B). The low probabilities of these species being part of the normal distribution suggested that there are at least two distinct $[Na]_{shoot}$ phenotypes among Caryophyllales species. The species with $[Na]_{shoot}$ at the limit for inclusion in the normal distribution were *Plumbago auriculata* (*P*=0.0153, rank #41), *Gomphrena serrata* (*P*=0.0106, rank #42), *Rumex hydrolapathum* (*P*=0.0003, rank #43) and *Limonium sinuatum* (*P*=0.0001, rank #44).

The distribution of log-normal $[Na]_{shoot}$ of Caryophyllales species appeared to comprise the sum of two discrete log-normal distributions (Figure 2B). The first log-normal distribution (mean = -0.3717, standard deviation = 0.3299, *n*=49 species) contained 49 species and the second log-normal distribution (mean = 1.246, standard deviation 0.2756, *n*=12 species) contained 12 species (Figure 2B). Since these two log-normal distributions differed significantly (P<0.0001), these data suggest that there are at least two distinct $[Na]_{shoot}$ phenotypes among Caryophyllales species. Considering the species with log $[Na]_{shoot}$ at the extremes of these two distributions, the log $[Na]_{shoot}$ of *Psylliostachys suworowi* had a greater probability of being in the first rather than the second log-normal distribution (*P*=0.0076 versus *P*=0.0017), whilst the log $[Na]_{shoot}$ of *Spergula arvensis* had a greater probability of being in the second rather than the first log-normal distribution (*P*=0.0210 versus *P*=0.0007). The trait of abnormally large $[Na]_{shoot}$ when plants are grown in non-saline solutions will, henceforth, be termed “Na-hyperaccumulation” and the discrete set of 12 Caryophyllales species with large log $[Na]_{shoot}$ were considered to be “Na-hyperaccumulators”.

The evolutionary origin of Na-hyperaccumulation was sought by comparing the number of Na-hyperaccumulator species and the mean $[Na]_{shoot}$ in different families of the Caryophyllales (Figure 3). The 12 Caryophyllales species exhibiting Na-hyperaccumulation were distributed across five of the ten Caryophyllales families represented in this study.

However, the trait was most prevalent in the Aizoaceae. Six of the seven Aizoaceae species studied exhibited Na-hyperaccumulation. These six species were among the seven Caryophyllales species with the largest $[Na]_{shoot}$ (Table S1). Consequently, the Aizoaceae had the largest mean $[Na]_{shoot}$ (24.47 ± 5.07 mg g^{-1} DM, *n*=7 species) of all the Caryophyllales families. The only Cactaceae species studied, *Echinofossulocactus* sp., also had one of the largest $[Na]_{shoot}$ measured (17.60 mg g^{-1} DM, *n*=1 experiment). In addition, two of the twelve Amaranthaceae species studied (*Atriplex hortensis*, *Beta vulgaris*), two of the twenty Caryophyllaceae species studied (*Silene armeria*, *Spergula arvensis*) and one of the two Portulacaceae species studied (*Portulaca grandiflora*) could also be considered Na-hyperaccumulators (Table S1). However, since (1) there were proportionally fewer Na-hyperaccumulator species in these families and (2) the Na-hyperaccumulator species in these families generally had smaller $[Na]_{shoot}$ than the Aizoaceae Na-hyperaccumulator species, their mean $[Na]_{shoot}$ was less than the mean $[Na]_{shoot}$ of the Aizoaceae (Figure 3). No Na-hyperaccumulator species were observed in the Phytolaccaceae, Nyctaginaceae, Montiaceae, Polygonaceae or Plumbaginaceae. Based on the phylogenetic relationships between Caryophyllales families proposed recently (Crawley & Hilu, 2012; Hernández-Ledesma *et al.*, 2015; Yang *et al.*, 2015) and the data from the experiments reported here (Table S1) it appears that the trait of Na-hyperaccumulation might have evolved several times within the Caryophyllales (Figure 3). It is likely that the trait evolved in an ancestor of the Aizoaceae, but not the Phytolaccaceae or Nyctaginaceae. It is possible that the trait also evolved in ancestors of the Cactaceae and Portulacaceae, which are closely related (APGIV, 2016), and in ancestors of the Amaranthaceae and Caryophyllaceae.

Discussion

Angiosperm species can be classified into “Na-excluders”, “Na-responders” and “Na-accumulators” based on relationships between their $[Na]_{shoot}$ and the salinity of the irrigation solution (cf. Baker, 1981). This terminology, originally proposed to categorise the responses of plant species to toxic elements (“heavy metals”) in the environment, also appears valid for Na accumulation, since the accumulation of excessive Na^+ can be toxic to plants and plant species respond to Na^+ in their environment either by excluding this cation

409 or accumulating it safely in their tissues. Only two of the ten halophytic species studied in
410 detail in this paper could be classified as Na-accumulators (Table 1). These were the
411 Caryophyllales species *Atriplex hortensis* and *Beta vulgaris*, which both had exceptionally
412 large $[Na]_{shoot}$ when irrigated with either non-saline or saline solutions. A similar response of
413 $[Na]_{shoot}$ to increasing salinity in the root environment has been observed previously for
414 other Caryophyllales species including members of the *Atriplex*, *Salicornia* and *Suaeda*
415 genera (Albert, 1982; Glenn & O'Leary, 1984). However, not all Caryophyllales species
416 exhibit this trait and the response of $[Na]_{shoot}$ to increasing salinity in the root environment
417 of, for example, the miohalophytes *Rumex dentatus* and *Limonium perezii* is typical of a Na-
418 excluders whilst the response of $[Na]_{shoot}$ to increasing salinity in the root environment of,
419 for example, *Sarcobatus vermiculatus* is reminiscent of Na-responders (Glenn & O'Leary,
420 1984).

421 The prevalence of Na-accumulator species, which exhibit abnormally large $[Na]_{shoot}$
422 when grown under non-saline conditions, was assessed by combining data from six
423 glasshouse experiments in which 334 angiosperm species representing 35 angiosperm
424 orders had been grown hydroponically in a non-saline solution containing 0.1 mM Na for 18-
425 73 days (Table S1). It was observed that a relatively large number of Caryophyllales species
426 exhibited abnormally large $[Na]_{shoot}$ (>10 mg g^{-1} DM) when grown in non-saline solutions
427 (Table S1; Figure 1). The distribution of the log-normal $[Na]_{shoot}$ of Caryophyllales species
428 appeared to comprise two discrete log-normal distributions containing 49 and 12 species,
429 respectively (Figure 2), suggesting that there are at least two distinct $[Na]_{shoot}$ phenotypes
430 among Caryophyllales species. The $[Na]_{shoot}$ distinguishing between these two distributions
431 was about 4 mg Na g^{-1} DM.

432 The ability of plants to accumulate Na when growing in non-saline environments is
433 not considered to be an evolutionary advantage (Cheeseman, 2015). Indeed, it has been
434 suggested that grazing by herbivores has selected for glycophyte species that maintain
435 $[Na]_{shoot}$ below about 1-2 mg g^{-1} DM (Cheeseman, 2015). Nevertheless, it is possible that the
436 ability to accomodate large $[Na]_{shoot}$ might be an enabling trait allowing species to adapt to a
437 variety of abiotic environmental challenges. It might confer the ability for osmotic
438 adjustment in environments with low K phytoavailability or contribute to tolerance of arid
439 or saline environments (Flowers & Colmer, 2008; Kadereit *et al.*, 2012; White, 2013).
440 However, it can be observed that the trait of Na-hyperaccumulation within the

Caryophyllales is not directly correlated with the expression of either C_4 photosynthesis or CAM, tissue succulence, halophytism in general, or the euhalophytic trait in particular (Table S2).

The evolutionary origins of the trait of abnormally large shoot Na accumulation when plants are grown in non-saline solutions, termed "Na-hyperaccumulation", can be investigated by mapping this trait on the phylogenetic relationships between Caryophyllales families (Crawley & Hilu, 2012; Hernández-Ledesma *et al.*, 2015; Yang *et al.*, 2015). All Aizoaceae species appear to exhibit Na-hyperaccumulation when grown in non-saline environments (Table S2). Although *Delosperma cooperi* was not classified as a Na-hyperaccumulator in the present study, it has previously been shown to accumulate >4 mg Na g^{-1} DM shoot when grown in a peat substrate (Sunshine Mix #1, SunGro Hort., Bellevue, Washington) and irrigated with tap water with an EC_e of 0.8 dS m^{-1} (Niu & Rodriguez, 2006). In addition to the species studied in the present study, *Galenia pubescens* (Patel *et al.*, 1980), *Galenia secunda* (Glenn & O'Leary, 1984), *Sesuvium portulacastrum* (Ramani *et al.*, 2006; Slama *et al.*, 2008; Rabhi *et al.*, 2011; Wang *et al.*, 2012), *Sesuvium verrucosum* (Glenn & O'Leary, 1984) and *Tetragonia tetragonioides* (Yousif *et al.*, 2010) have all been reported to accumulate >4 mg Na g^{-1} DM shoot when grown under non-saline conditions (Table S2). In this context, it is noteworthy that many Aizoaceae species possess bladder cells (Thomson *et al.*, 1988; Flowers *et al.*, 2010).

The trait of Na-hyperaccumulation in non-saline environments is less ubiquitously exhibited by Amaranthaceae species (Table S2 and references therein). However, it is exhibited by many species formerly classified as Chenopodiaceae. It is exhibited by the Betoideae, *Beta vulgaris* and *Hablitzia tamnoides*, by some Camphorosmoideae (e.g. *Bassia hyssopifolia* and *Maireana brevifolia*), by many Chenopoidioideae including most, but not all, *Atriplex* and *Chenopodium* species, by *Corispermum hyssopifolium* and *Corispermum pallasii* subsp. *membranaceum*, by all the Salicornioideae studied, including several *Salicornia* and *Tecticornia* species, by many Salsoloideae, and all *Suaeda* species (Table S2). By contrast, the trait is not exhibited by any Amaranthaceae *sensu stricto* (Amaranthoideae, Gomphrenoideae), with the exception of *Ptilotus polystachyus*. Although many Amaranthaceae species possess bladder cells or salt glands (Thomson *et al.*, 1988; Fahn & Cutler, 1992; Flowers *et al.*, 2010; LoPresti, 2014), there does not appear to be a direct

correlation between the presence of salt glands and the ability to hyperaccumulate Na in non-saline environments (Table S2).

Few Caryophyllaceae species had large $[Na]_{shoot}$ when grown hydroponically in non-saline solutions, with only two of the twenty species examined in the present study (*Silene armeria*, *Spergula arvensis*) exhibiting Na-hyperaccumulation (Table S1; Figure 1). This is consistent with previous studies (Sonneveld & Voogt, 1983; Kwon *et al.*, 2005; Heo *et al.*, 2007; Jeong *et al.*, 2014). Several species in the Sarcobataceae (*Sarcobatus vermiculatus*), Portulacaceae (*Portulaca grandiflora*; *Portulaca oleracea*) and Cactaceae (*Carnegiea gigantea*, *Echinocactus grusonii*, *Echinofossulocactus* sp., *Opuntia ficus-indica*) exhibit large $[Na]_{shoot}$ when grown in non-saline environments (Table S2 and references therein). However, it is clear from the literature that not all Cactaceae exhibit large $[Na]_{shoot}$ when grown in non-saline environments (Table S2; Nobel, 2003; Goodman *et al.*, 2012). No species in the Phytolaccaceae, Nyctaginaceae, Montiaceae, Basellaceae or Simmondsiaceae exhibited the trait (Table S2 and references therein).

In the experiments reported here, no Na-hyperaccumulator species were observed in the Plumbaginaceae or Polygonaceae (Table S1; Figure 3). Nevertheless, several species in these families have been reported to accumulate large $[Na]_{shoot}$ when grown in non-saline environments (Table S2 and references therein). In addition, all six species of Tamaricaceae studied to date appear to accumulate large $[Na]_{shoot}$ when grown in non-saline environments (Patel *et al.*, 1980; Ding *et al.*, 2010; Li *et al.*, 2010; Gorai & Neffati, 2011; Sghaier *et al.*, 2015; Sharif & Khan, 2016). It is, perhaps, noteworthy that many species in the Plumbaginaceae and Tamaricaceae possess salt glands, whilst members of the Polygonaceae do not (Thomson *et al.*, 1988; Fahn & Cutler, 1992; Salama *et al.*, 1999; Flowers *et al.*, 2010). Again, there does not appear to be a direct correlation between the occurrence of salt glands and the ability of a species to hyperaccumulate Na in non-saline environments (Table S2).

In conclusion, phylogenetic relationships between Caryophyllales families suggest that the trait of Na-hyperaccumulation in non-saline environments has evolved several times within this order (Figure 3). The data presented here suggest that the trait evolved in an ancestor of the Aizoaceae, but not the Phytolaccaceae or Nyctaginaceae. It is also likely that the trait also evolved in an ancestor of species formerly classified as Chenopodiaceae (subfamilies Betoideae, Chenopodioideae, Camphorosmoideae, Salsoloideae,

Salicornioideae, Suaedoideae), but not the Amaranthaceae *sensu stricto* (subfamilies Amaranthoideae, Gomphrenoideae). In addition, it is possible that the trait evolved in ancestors of the Sarcobataceae, Portulacaceae, Cactaceae, Tamaricaceae, Plumbaginaceae, and Polygonaceae, but further studies are required to explore these hypotheses. Future studies should focus on elucidating the evolutionary origin of Na-hyperaccumulation in non-saline environments (1) among species formerly classified as Chenopodiaceae, (2) among species in the Cactaceae and Portulacaceae, which are currently underrepresented in published studies, and (3) among species in the Plumbaginaceae, Tamaricaceae and Polygonaceae, to determine the extent of the trait in these families.

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706
707
708 **Supporting Information**
709
710 Additional supporting information may be found in the online version of this article.
711
712 **Table S1** Shoot sodium concentrations in 334 species from 35 angiosperm orders grown
713 hydroponically in a non-saline solution containing 0.1 mM Na^+ in at least one of six
714 glasshouse experiments.
715
716 **Table S2** Occurrence of sodium (Na)-hyperaccumulator species, having shoot Na
717 concentrations >4 mg g^{-1} dry matter when grown in non-saline environments, within the
718 Caryophyllales order, together with their halophytic and photosynthetic characteristics.
719

720 **Table**

721

722 **Table 1** Shoot fresh weight, dry matter (DM) and sodium concentration ($[Na]_{shoot}$) of ten
723 halophytic angiosperm species grown in pots irrigated with either 100 mL non-saline (0.14
724 mM Na) or saline (50-300 mM Na) solution per week. The experiment was initiated by
725 increasing the NaCl concentration in the irrigation water of the saline treatment to 50 mM
726 for the first week, then 150 mM NaCl for the second week, and finally 300 mM for the third
727 week. Plants were harvested three weeks after the first addition of NaCl to the saline
728 irrigation water. Data are expressed as mean ± standard error of the mean of n
729 observations.

730

Treatment	Species	Family	Order	Fresh Weight (g)	Dry Matter (g)	$[Na]_{shoot}$ (mg g^{-1} DM)
Non-saline	*Hordeum jubatum* L.	Poaceae	Poales	4.66 ± 2.24 (*n*=3)	0.43 ± 0.33 (*n*=3)	0.21 ± 0.02 (*n*=3)
Non-saline	*Asparagus officinalis* L.	Asparagaceae	Asparagales	6.36 ± 0.75 (*n*=10)	0.74 ± 0.18 (*n*=10)	0.38 ± 0.05 (*n*=10)
Non-saline	*Hibiscus tilliaceus* L.	Malvaceae	Malvales	2.87 (*n*=1)	0.19 (*n*=1)	0.69 (*n*=1)
Non-saline	*Colubrina asiatica* (L.) Brogn.	Rhamnaceae	Rosales	1.47 ± 0.17 (*n*=2)	0.056 ± 0.003 (*n*=2)	0.73 ± 0.15 (*n*=2)
Non-saline	*Casuarina cunninghamiana* Miq.	Casuarinaceae	Fagales	0.90 ±0.20 (*n*=2)	0.045 ± 0.010 (*n*=2)	1.03 ± 0.24 (*n*=2)
Non-saline	*Kosteletzkya virginica* (L.) C. Presl ex A. Gray	Malvaceae	Malvales	22.69 ± 3.75 (*n*=9)	2.09 ± 0.63 (*n*=9)	1.62 ± 0.13 (*n*=9)
Non-saline	*Ammi visnaga* (L.) Lam.	Apiaceae	Apiales	22.56 ± 1.52 (*n*=8)	2.13 ± 0.18 (*n*=8)	2.16 ± 0.11 (*n*=8)
Non-saline	*Lobularia maritima* (L.) Desv.	Brassicaceae	Brassicales	20.45 ± 8.68 (*n*=4)	1.10 ± 0.58 (*n*=4)	3.40 ± 0.27 (*n*=4)
Non-saline	*Scaevola crassifolia* Labill.	Goodeniaceae	Asterales	49.53 ± 3.92 (*n*=4)	4.09 ± 0.27 (*n*=4)	3.78 ± 0.46 (*n*=4)
Non-saline	*Plantago maritima* L.	Plantaginaceae	Lamiales	3.32 ± 0.52 (*n*=12)	0.053 ± 0.001 (*n*=12)	4.11 ± 0.33 (*n*=12)
Non-saline	*Beta vulgaris* L.	Amaranthaceae	Caryophyllales	38.95 ± 7.28 (*n*=6)	2.46 ± 0.63 (*n*=6)	10.72 ± 1.03 (*n*=6)
Non-saline	*Atriplex hortensis* L.	Amaranthaceae	Caryophyllales	28.18 ± 1.52 (*n*=7)	4.18 ± 0.47 (*n*=7)	12.02 ± 0.49 (*n*=7)
Saline	*Hordeum jubatum* L.	Poaceae	Poales	2.52 ± 0.86 (*n*=2)	0.13 ± 0.07 (*n*=2)	2.18 ± 0.27 (*n*=2)
Saline	*Asparagus officinalis* L.	Asparagaceae	Asparagales	4.11 ± 0.66 (*n*=9)	0.36 ± 0.15 (*n*=9)	2.66 ± 0.97 (*n*=9)
Saline	*Hibiscus tilliaceus* L.	Malvaceae	Malvales	3.36 (*n*=1)	0.17 (*n*=1)	4.10 (*n*=1)
Saline	*Colubrina asiatica* (L.) Brogn.	Rhamnaceae	Rosales	1.19 ± 0.36 (*n*=2)	0.054 ± 0.003 (*n*=2)	16.73 ± 10.32 (*n*=2)
Saline	*Casuarina cunninghamiana* Miq.	Casuarinaceae	Fagales	0.53 (*n*=1)	0.060 (*n*=1)	3.62 (*n*=1)
Saline	*Kosteletzkya virginica* (L.) C. Presl ex A. Gray	Malvaceae	Malvales	15.02 ± 1.58 (*n*=9)	1.66 ± 0.26 (*n*=9)	13.60 ± 0.94 (*n*=9)
Saline	*Ammi visnaga* (L.) Lam.	Apiaceae	Apiales	20.38 ± 1.65 (*n*=8)	1.96 ± 0.22 (*n*=8)	17.83 ± 1.14 (*n*=8)
Saline	*Lobularia maritima* (L.) Desv.	Brassicaceae	Brassicales	9.54 ± 1.75 (*n*=4)	0.49 ± 0.18 (*n*=4)	27.94 ± 2.30 (*n*=4)
Saline	*Scaevola crassifolia* Labill.	Goodeniaceae	Asterales	41.70 ± 9.77 (*n*=4)	3.41 ± 0.97 (*n*=4)	19.41 ± 1.97 (*n*=4)
Saline	*Plantago maritima* L.	Plantaginaceae	Lamiales	2.47 ± 0.37 (*n*=12)	0.052 ± 0.002 (*n*=12)	27.49 ±1.01 (*n*=12)
Saline	*Beta vulgaris* L.	Amaranthaceae	Caryophyllales	35.72 ± 8.90 (*n*=6)	2.27 ± 0.60 (*n*=6)	28.08 ± 3.29 (*n*=5)
Saline	*Atriplex hortensis* L.	Amaranthaceae	Caryophyllales	32.99 ± 0.57 (*n*=6)	5.04 ± 0.24 (*n*=6)	35.70 ± 3.45 (*n*=3)

731

732 **Figure Legends**

733

734 **Figure 1** Frequency distributions of mean shoot sodium (Na) concentrations in **(a)** 334
735 species from 35 angiosperm orders or **(b)** 61 species from ten Caryophyllales families grown
736 hydroponically in a non-saline solution. Shoot Na concentrations >10 mg Na g^{-1} dry matter
737 are designated "more". The solid line indicates the normal (mean = 0.393, standard
738 deviation = 0.185 mg Na g^{-1} dry matter, *n* = 42 species) distribution fitted to data from the
739 42 Caryophyllales species with the smallest shoot Na concentrations.

740

741 **Figure 2** Frequency distributions of log-normal mean shoot sodium (Na) concentrations in
742 **(a)** 334 species from 35 angiosperm orders or **(b)** 61 species from ten Caryophyllales families
743 grown hydroponically in a non-saline solution. The solid line indicates two log-normal
744 distributions (first: mean = -0.3717, standard deviation = 0.3299, *n* = 49 species; second:
745 mean = 1.246, standard deviation 0.2756, *n* = 12 species) fitted to data from the 49
746 Caryophyllales species with the smallest leaf Na concentrations and the 12 Caryophyllales
747 species with the largest leaf Na concentrations, respectively.

748

749 **Figure 3** Phylogenetic relationships between ten families of the Caryophyllales, based on the
750 phylogeny derived by Crawley & Hilu (2012), and their shoot sodium concentrations
751 ($[Na]_{shoot}$). The number of species hyperaccumulating Na (numerator) and the number of
752 species surveyed (denominator) are indicated in parentheses. Families with species
753 expressing the trait of Na-hyperaccumulation are highlighted in yellow and Families without
754 species expressing the trait of Na-hyperaccumulation are highlighted in blue. Data are
755 expressed as mean values with capped lines indicating the standard error of the mean of
756 species surveyed.

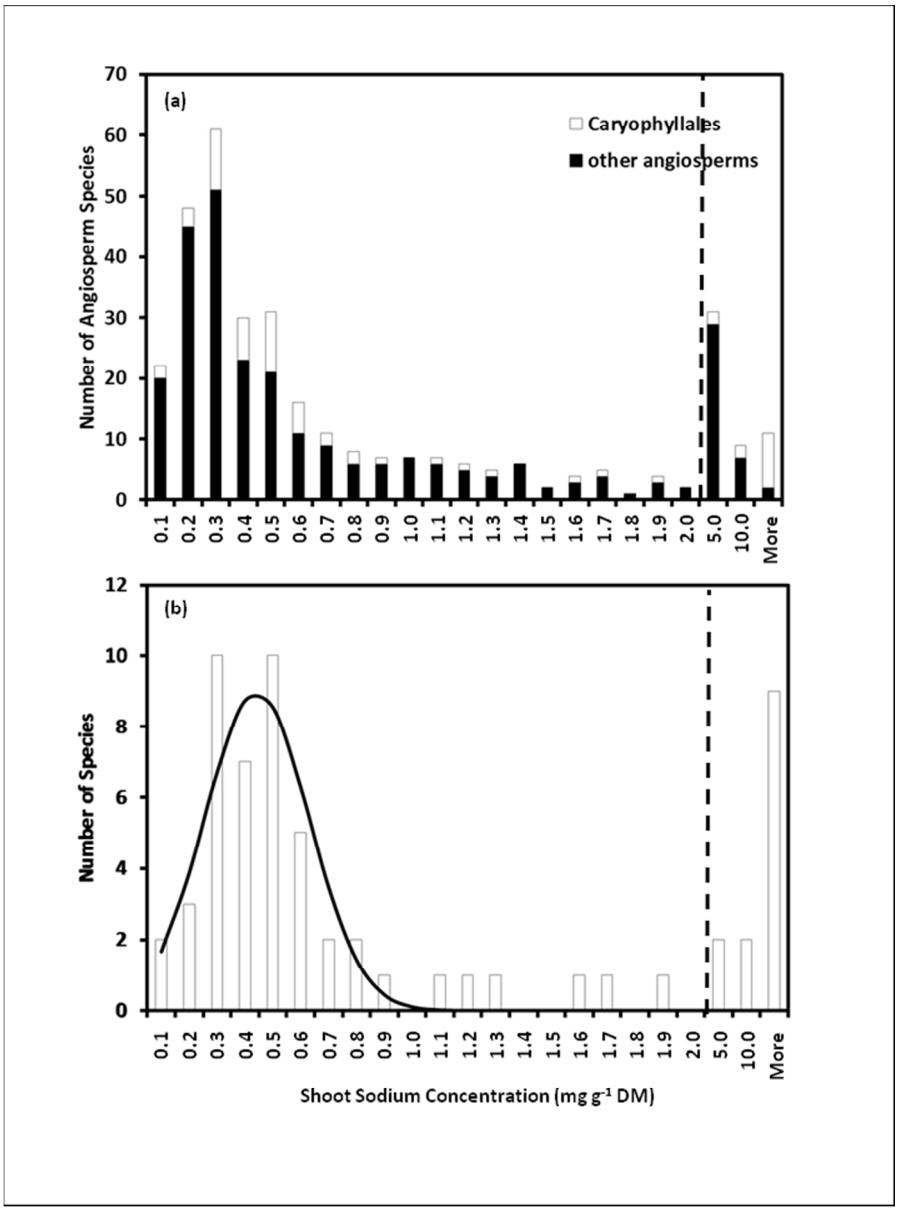


Figure 1 Frequency distributions of mean shoot sodium (Na) concentrations in (a) 334 species from 35 angiosperm orders or (b) 61 species from ten Caryophyllales families grown hydroponically in a non-saline solution. Shoot Na concentrations >10 mg Na g-1 dry matter are designated "more". The solid line indicates the normal (mean = 0.393, standard deviation = 0.185 mg Na g-1 dry matter, n = 42 species) distribution fitted to data from the 42 Caryophyllales species with the smallest shoot Na concentrations.

Fig. 1

120x162mm (150 x 150 DPI)

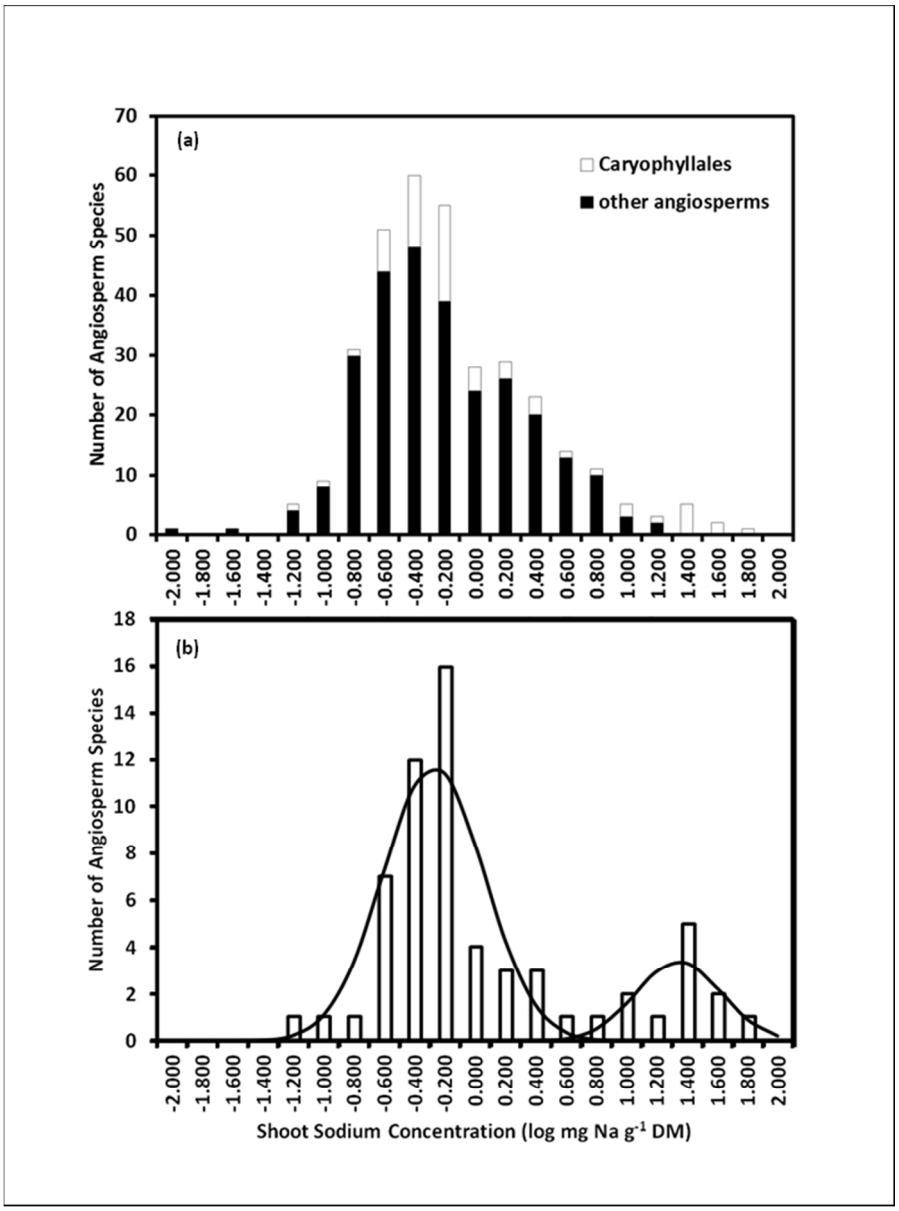


Figure 2 Frequency distributions of log-normal mean shoot sodium (Na) concentrations in (a) 334 species from 35 angiosperm orders or (b) 61 species from ten Caryophyllales families grown hydroponically in a non-saline solution. The solid line indicates two log-normal distributions (first: mean = -0.3717, standard deviation = 0.3299, n = 49 species; second: mean = 1.246, standard deviation 0.2756, n = 12 species) fitted to data from the 49 Caryophyllales species with the smallest leaf Na concentrations and the 12 Caryophyllales species with the largest leaf Na concentrations, respectively.

Fig. 2

120x162mm (150 x 150 DPI)

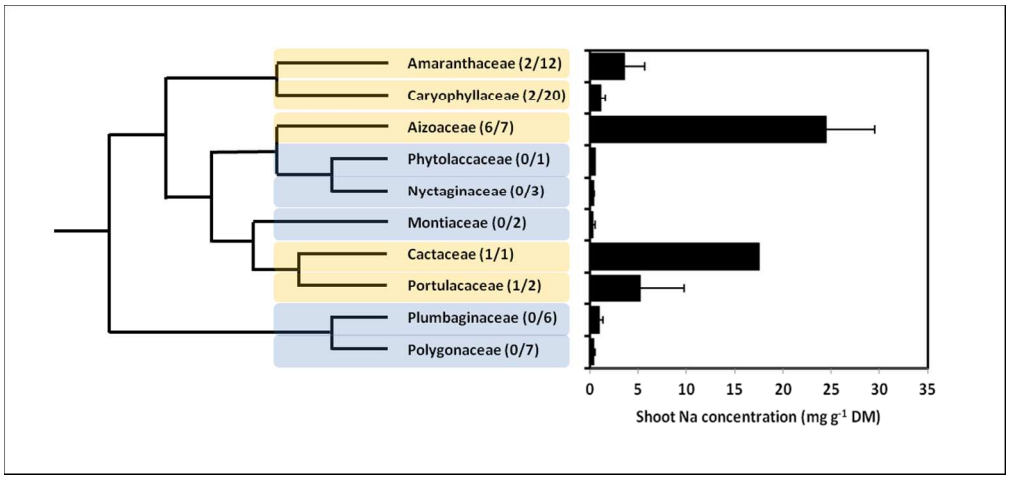


Figure 3 Phylogenetic relationships between ten families of the Caryophyllales based on Crawley & Hilu (2012) and their shoot sodium concentrations ([Na]shoot). The number of species hyperaccumulating Na (numerator) and the number of species surveyed (denominator) are indicated in parentheses. Data are expressed as mean values with capped lines indicating the standard error of the mean of species surveyed.
Fig. 3
215x101mm (150 x 150 DPI)

Figure 1

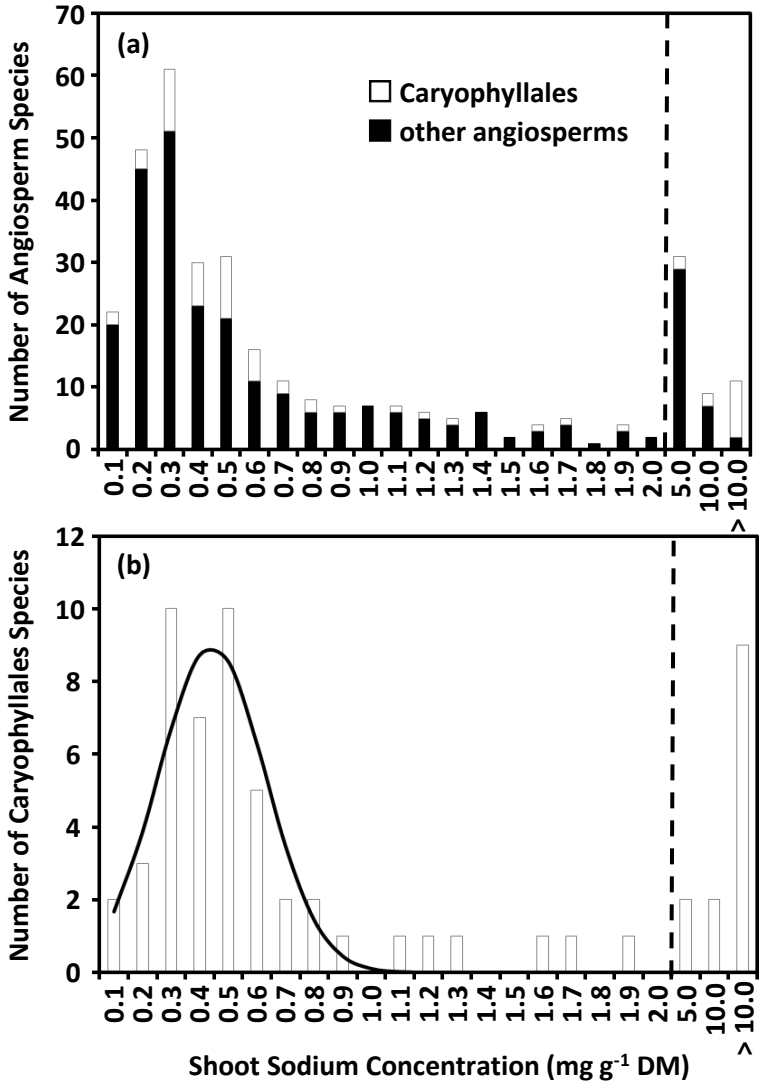

(a)
Caryophyllales
other angiosperms
Number of Angiosperm Species
0
10
20
30
40
50
60
70
(b)
Number of Caryophyllales Species
0
2
4
6
8
10
12
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0
5.0
10.0
> 10.0
Shoot Sodium Concentration (mg g-1 DM)

Figure 2

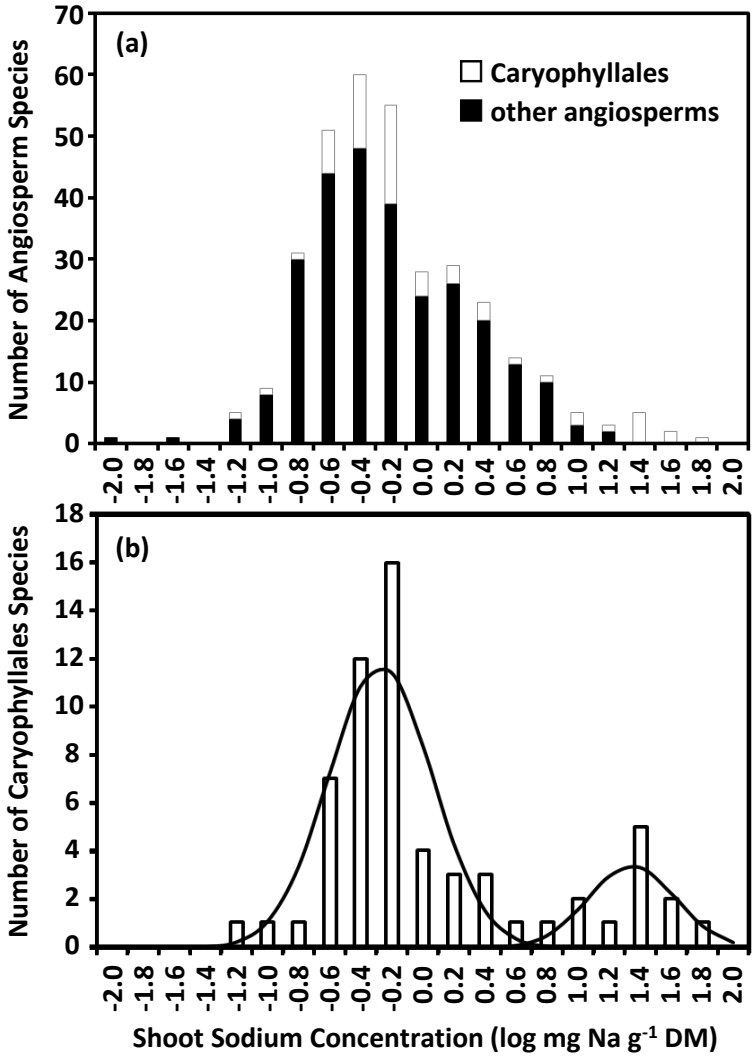
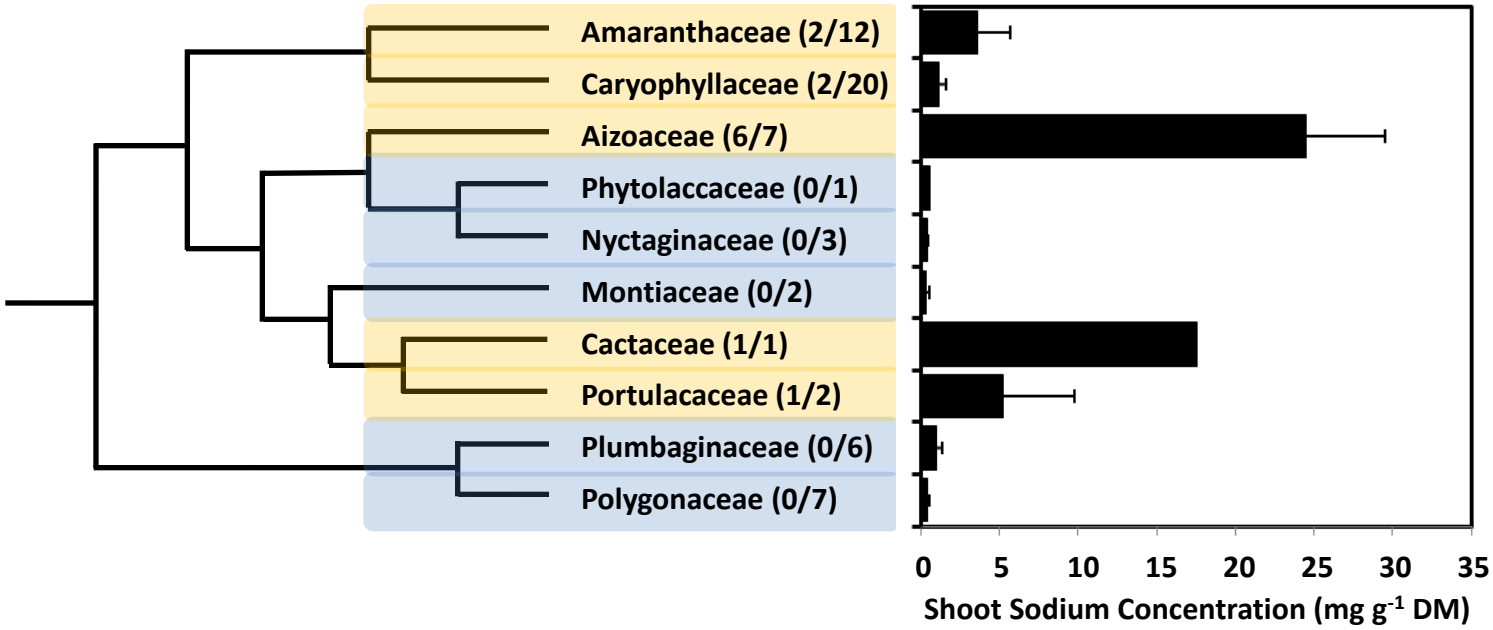

(a)
Caryophyllales
other angiosperms
Number of Angiosperm Species
70
60
50
40
30
20
10
0
(b)
Number of Caryophyllales Species
18
16
14
12
10
8
6
4
2
0
-2.0 -1.8 -1.6 -1.4 -1.2 -1.0 -0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0
Shoot Sodium Concentration (log mg Na g-1 DM)

Figure 3


Amaranthaceae (2/12)
Caryophyllaceae (2/20)
Aizoaceae (6/7)
Phytolaccaceae (0/1)
Nyctaginaceae (0/3)
Montiaceae (0/2)
Cactaceae (1/1)
Portulacaceae (1/2)
Plumbaginaceae (0/6)
Polygonaceae (0/7)
0
5
10
15
20
25
30
35
Shoot Sodium Concentration (mg g-1 DM)