Resurrection approach shows evolutionary changes in drought responses in European plant species after two decades of climate change

Robert Rauschkolb[1,*], Lisa Henres[1], Caroline Lou[2], Sandrine Godefroid[3], Lara Dixon[4], Oliver Bossdorf[1], Andreas Ensslin[5], J.F. Scheepens[1,6]

[1] Plant Evolutionary Ecology, Institute of Evolution and Ecology, University of Tübingen, Auf der Morgenstelle 5, 72076 Tübingen, Germany
[2] Department of Biology, McGill University, Montreal, Quebec, H3A 1B1 Canada
[3]Meise Botanic Garden, Nieuwelaan 38, 1860 Meise, Belgium
[4]Conservatoire Botanique National Méditerranéen de Porquerolles, 34 avenue Gambetta, 83400 Hyères, France
[5] Conservatory and Botanic Garden of the City of Geneva, 1296 Chambésy, Geneva, Switzerland
[6] Plant Ecology, Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany
* Corresponding author: robert.rauschkolb@uni-tuebingen.de, +49 7071 2974248
ORCID IDs: R.R. 0000-0002-8569-8705; S.G. 0000-0002-1893-9249; O.B. 0000-0001-7504-6511; A.E. 0000-0003-2922-5310; J.F.S. 0000-0003-1650-2008

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Abstract

Plant populations must continuously adapt to the impacts of ongoing global climate change, including warmer temperatures and more extreme weather events. We can detect such evolutionary changes within plant populations through the resurrection approach whereby plants grown from seeds stored in seed banks (“ancestors”) are compared to freshly collected seeds from the same populations (“descendants”) in common garden experiments. In this study we used the resurrection approach in two multi-species experiments to investigate changes in phenotypic traits and drought tolerance of European plant species from two biogeographic regions. In the seedling survival experiment using seedlings of four Mediterranean species, watering was ceased and day of mortality recorded. We found that descendants survived significantly longer without any watering but these seedlings were smaller than the ancestral seedlings. In the watering response experiment we investigated phenotypic responses to drought in adult plants of nine species originating from temperate climatic regions in Europe. We found that descendant plants were significantly taller under well-watered conditions but smaller under drought than their ancestors, thus showing stronger plasticity. Our study suggests that plants have already evolved phenotypically, including through changes in trait means and plasticity, within the last decades. The observed evolutionary changes are consistent with adaptation to increased drought. More generally, the resurrection approach proved to be a useful tool to study rapid evolutionary processes in plants under climate change. Future studies should include fitness measures and comparative transplantations of descendants and ancestors into

their original habitat to disentangle adaptive from non-adaptive responses to recent climate change.

Introduction

Climate change has increased dramatically over the last decades (IPCC 2018), and plant populations are already responding (Peñuelas and Filella 2001, Parmesan and Yohe 2003). During the 21st century annual precipitation sums will further increase in northern Europe and decrease in the south (IPCC 2013). For central and western Europe, precipitation is expected to increase in winter and decrease in summer (IPCC 2013), leading to more droughts in the growing season. Moreover, the anticipated higher temperatures will lead to higher evapotranspiration (Feng and Fu 2013). These changes in environmental conditions will likely increase the frequency, duration and geographic extent of drought events in Southern and Central Europe (Ruosteenoja et al 2018; Samaniego et al 2018; Spinoni et al 2018). Changes in water availability and more frequent droughts are especially strong stressors for plants (Jaleel et al 2009; Fleta-Soriano and Munné-Bosch 2016), and many plant populations may not be well enough adapted to these novel environmental conditions (Anderson et al 2012; Shaw and Etterson 2012). To avoid extinction, plant populations need to migrate to track suitable conditions and so they may respond through phenotypic plasticity or adaptive evolution, or both (Holt 1990; Hoffmann and Sgrò 2011). For populations to survive drought events *in situ*, they may plastically or genetically adjust functional traits, which could involve reduced growth (Kusaka et al 2005; Shao et al 2008), increased root-shoot-ratio (Sharp and LeNoble 2002; Aroca 2012) or changes in the ratio of chlorophyll a/b and carotenoids (Farooq et al 2009).

Since drought events are periodic, the ability to change functional trait values through phenotypic plasticity may be a better strategy than to evolve constitutive changes in mean traits (Sultan and Spencer 2002, Alpert and Simms 2002, Gianoli and Valladares 2012), especially in environments with strong climatic variability. Studies on the effects of climate change on plant populations often only consider changes in mean climate conditions (Bertrand et al 2011), but there is strong evidence for increased climatic variability – specifically more heavy rain events followed by longer dry periods in many regions (Kharin et al 2007). It is known that changes in environmental variability may constitute distinct selection pressures and result in adaptive evolution of plasticity. For example, Lázaro-Nogal et al (2015) showed in a common garden study with several origins of *Senna candolleana* that populations originating from climatically more variable heterogeneous environments had a higher plasticity in growth traits. A similar observation was made by Gianoli and Gonzáles-Teuber (2005) who showed that plasticity in leaf area, leaf shape, leaf area ratio (LAR), and foliar trichome density in *Convolvulus chilensis* was highest for plants from the population with the highest interannual variation in precipitation. Thus, increased climatic variability appears to be associated with systematic, and presumably adaptive, changes in phenotypic plasticity. The fate of plant populations will also depend on their ability to adapt to altered climatic variability.

A powerful method to test for recent evolution - whether in trait means or in their plasticity - is to compare ancestors with their descendants by using stored propagules such as seeds (Orsini et al 2013; Merilä and Hendry 2014; Franks et al 2018). If ancestors can be revived, the resulting plants can be compared to individuals raised from propagules sampled from the same population today. Growing ancestors and descendants together under common conditions then allows to directly test for heritable trait differentiation among temporally separated populations (Franks et al 2007, 2008). Understanding how populations responded evolutionarily in the past is extremely valuable for making predictions for future population responses to environmental change (Orsini et al 2013; Franks et al 2018).

An increasing number of studies have used this so-called “resurrection approach” to compare plants grown from seeds collected before and after drought events. They have shown that plants adapted their phenology through an early start of flowering in order to avoid temporary droughts (Franks et al 2007; Nevo et al 2012; Vigouroux et al 2011; Thomann et al 2015). For growth traits, results appear to be more species-specific. For example, in an experiment with *Mimulus laciniatus* by Dickmann (2016) drought-adapted plants grew bigger, whereas Vigouroux and colleagues (2011) showed opposite results in a study with *Pennisetum glaucum*.

Here, we investigated whether single populations of multiple plant species from Mediterranean and temperate regions of Europe have already evolved their drought tolerance over the last decades as the expected response to more frequent and longer drought events (Met office 2011; DWD 2018; IRM 2020). To investigate this, we conducted two common garden experiments in which we applied drought treatments to plants raised from seeds stored for at least 20 years in three different seed banks (ancestors) and from seeds that we collected from the same populations in 2018 (descendants). In the first experiment (“seedling survival experiment”), we used four Mediterranean species to test whether seedlings of the descendants survived longer without watering than the seedlings of their ancestors. Seedling establishment is a crucial process for population survival (Grubb 1977), and seedlings are especially susceptible to drought (Moles and Westoby 2004). Therefore seedling drought resistance should be under high selection pressure in increasingly dry and more variable environments (Schupp 1995; Fenner and Kitajima 1999). In the second experiment ("watering response experiment") we worked with adult plants from nine temperate European species. We subjected ancestors and descendants to well-watered vs. dry conditions and compared their growth responses to test the hypothesis that populations evolved phenotypic traits, and/or their plasticities, to cope with increased droughts.

Material and Methods

Seed collection

For the seedling survival experiment we obtained seeds of Mediterranean species from the seed bank at the Conservatoire Botanique National Méditerranéen de Porquerolles (CBNMed, Hyères, France). The seed material for the watering response experiment was provided by the Meise Botanic Garden (Belgium) and the botanical garden of Osnabrück (Germany). For both experiments, we only used seeds with precise sampling dates and location records, and which had been stored for at least 20 years. We only selected species with a short life cycle as they are expected to respond more quickly to selection and are therefore more likely to show rapid evolutionary changes between ancestors and descendants. Finally, we specifically chose species with relatively isolated (but large) populations to reduce outcrossing with other populations. From the CBNMed (seedling survival experiment) we used seeds of four species that met these requirements (Table 1): *Anthemis maritima, Matthiola tricuspidata, Medicago* *marina* and *Plantago subulata*. The seeds of these four species had been collected between 1992 and 1997 in the region of Hyères, Southern France, where average temperatures have increased by 1.5 °C and precipitation decreased during the last 30 years (Met office 2011). After ancestor seed collection, the seeds of *A. maritima*, *M. tricuspidata* and *M. marina* were cleaned, dried and stored at 5°C, whereas seeds of *Plantago subulata* were ultra-dessicated and stored at 17°C at the CBNMed until November 2018. For the watering response experiment we used seeds of a total of nine species that met our requirements (Table 1): *Centaurium erythraea*, *Clinopodium vulgare*, *Dianthus carthusianorum*, *Digitalis lutea*, *Leontodon hispidus*, *Melica ciliata*, *Pimpinella saxifraga*, *Sedum album*, *Teucrium chamaedrys*. The seeds of these nine species had been collected between 1992 and 1995 in Belgium and close to Osnabrück, Lower-Saxony (Germany). In Belgium, summer temperatures have increased by 0.37°C per decade since 1981 (IRM 2020) and in northern Germany mean annual temperature increased by 0.7°C when comparing 1961-1990 to 1981-2010 (DWD 2018). After ancestor seed collection, the seeds had been cleaned, dried at 15% relative humidity and then stored at -20°C at Meise Botanic Garden and the botanical garden of Osnabrück until November 2018. To obtain the descendants, seeds of all species were collected from the same populations in spring (Mediterranean species) and summer 2018 (temperate species).

Seedling survival experiment

In order to break physical seed dormancy, we scarified seeds of *M. marina* by softly scrubbing them with sandpaper. To reduce the growth of bacteria or fungi during germination, we surface-sterilized all seeds with 3% Sodium Hypochlorite (NaOCl) and two drops of Tween20 per 200ml solution, and washed them three times with sterilized water. We germinated all seeds on 1% water agar in 90 mm Petri dishes. After one week of cold-dark stratification at 5°C we transferred the Petri dishes to a walk-in growth chamber (light intensity = 230 $\mu mol*m^{-2}*s^{-1}$, 50% relative humidity) with a light/dark cycle of 8/16 hours and temperatures of 23/18°C.

For each species we filled one seedling tray (96-cell QuickPot®, 3.8 x 3.8 cm cells) with a standard potting soil (Einheitserde®, BioLine, Topfsubstrat Öko torffrei) and planted seedlings into every other cell so that seedlings did not grow directly next to each other. We planted the ancestors and descendants in an even pattern, but to identify the seedlings, we did not use any labels but noted their position separately from the tray in order to reduce observer bias. The trays were placed in a walk-in growth chamber with a light/dark cycle of 12/12 hours and 23/18°C (light intensity = 230 $\mu mol*m^{-2}*s^{-1}$, 50% relative humidity). To reduce insect damage, sticky traps were distributed throughout the room. The seedlings were watered regularly for 2-3 weeks (depending on the species) to allow their establishment, and after that, we stopped watering to simulate a temporary drought. We recorded mortality due to desiccation at least every other day. A seedling was scored as dead when it was completely dry and all leaves had lost their green colour. We cut each dead seedling 1 mm above ground, dried it at 60°C for 48 h, and weighed it.

We used linear models to analyse how seedling origin (ancestor vs. descendant) affected mean time to desiccation and aboveground biomass. To account for species differences, we included species identity in the model as a fixed factor. For the analyses of time to desiccation we included days between transplanting and start of the experiment and for the analyses of aboveground biomass the total lifespan of the seedling as covariables. We additionally analysed the data separately for each species, using the same model but excluding species identity. Finally, we used linear models to test whether the mean time to desiccation of a plant individual is explained by its aboveground biomass while correcting for the total lifespan of the seedling. For all models we checked the residuals visually for normality and heteroscedasticity.

Watering response experiment

We germinated seeds in trays filled with a standard potting soil (Einheitserde®, BioLine, Pikiersubstrat), with each origin sown into a separate tray. In order to break seed dormancy we kept the soil moist and cold-dark stratified the seeds at 5°C for two months. In March 2019, we transferred the trays to the greenhouse and allowed the seeds to germinate at 20°C and a natural spring daylight regime. We kept the seedlings in the trays for three months before the start of the experiment

We filled 9 x 9 cm pots with a 3:1 mixture of potting soil (Einheitserde®, BioLine, Topfsubstrat Öko torffrei) and sand (0-2 mm play sand, WECO GmbH). We transplanted the seedlings into the pots within one week in June 2019, and we always planted pairs of ancestor and descendant seedlings that were approximately of equal size. Right after transplantation we measured shoot length or rosette diameter (henceforth referred to as plant size) and the number of leaves or shoots, depending on the species (Table 1). After two weeks we separated all plants into a well-watered control group and a drought-suffering group, each with at least seven replicates per origin (ancestor vs. descendant) and species (Table 1). When five of the pots of a species had a dry soil surface, all plants of that species were watered, with control plants receiving 60 mL and drought plants receiving 30 mL water at each watering event. We re-randomized all pots in the greenhouse weekly. After eight weeks we repeated the growth trait measurements and subsequently harvested the plants and determined their aboveground biomass after oven-drying at 60°C for three days.

In order to be able to compare various measured traits across nine different species, we transformed all data by dividing the measured value for a trait by the mean value of this trait for a species. We then analysed the variation in plant size, number of leaves or shoots, and aboveground biomass with linear models that included origin (ancestor vs. descendant), treatment (drought vs. control) and species, and all possible interactions, as explanatory variables. A two-way interaction between plant origin and treatment would indicate that plants have evolved a different response to drought, and a three-way interaction between plant origin, treatment and species would show that species vary in their evolutionary responses to drought. We subsequently used linear models including only origin, treatment and their interaction to analyse the data for each species separately. As the sizes of transplanted seedling differed, we corrected for this by including the initial size measurement as a covariate in all our models. For all models we visually checked the residuals for normality and heteroscedasticity.

All analyses were done in R using the packages *plyr* (Wickham 2011) and *ggplot2* (Wickham 2016). All data is accessible as supplements in Tables S1 and S2.

Results

Seedling survival experiment

In the seedling survival experiment, seedlings from descendants survived on average almost two days longer than seedlings from ancestors (Fig.1a, $F_{1,208} = 12.99$, $p < 0.001$). We also found species differences in mean survival ability (Fig. 1a, $F_{3,208} = 255.21$, $p < 0.001$) and an interaction between species and origin (ancestors vs. descendants; Fig. 1a, $F_{3,208} = 2.74$, $p = 0.04$). The overall effect of temporal origin is strongly influenced by a single species, *A. maritima*, since only descendants from this species showed a significantly longer survival than their ancestors in individual species analyses (Fig. 1a, $F_{1,60} = 6.01$, $p = 0.017$). On average, seedlings from descendants had a significantly lower biomass than those from ancestors (Fig. 1b, $F_{1,204} = 19.92$, $p < 0.001$). Again, there was an interaction between species and origin (Fig. 1b, $F_{3,204} = 3.57$, $p = 0.015$), with the overall effect largely driven by *A. maritima* as only this species showed a significant biomass difference between origins in individual species analyses (Fig. 1b, $F_{1,59} = 6.08$, $p = 0.016$). Across species, plants with a lower biomass generally survived longer ($F_{1,200} = 12.43$, $p = <0.001$, $r^2=0.46$). However, at the species level we observed such a significant negative correlation between biomass and survival only for *A. maritima* ($F_{4,58} = 4.03$, $p = 0.006$, $r^2=0.16$), whereas for *M. tricuspidata* ($F_{3,59} = 4.01$, $p = 0.012$, $r^2=0.13$) and *M. marina* ($F_{4,46} = 7.71$, $p <0.001$, $r^2=0.35$) there were positive correlations, i.e. larger plants survived longer.

Watering response experiment

In the watering response experiment, the drought treatment had a significant effect on all three measured traits. Across all nine species, plants grown under drought conditions grew smaller, produced less branches or leaves and had a lower aboveground biomass (Fig. 2a-c). These observations were also consistent at the species level: wherever a significant effect occurred, the drought treatment lowered plant performance (Table 2). There were no significant interactions between species and treatment. The temporal origin did not affect plant size (shoot length and rosette diameter) in any of the studied species, but we found a significant difference in aboveground biomass between the ancestors and descendants of three species: In *C. erythraea* and *M. ciliata* ancestors produced significantly less biomass, whereas in *D. carthusianorum* the opposite was true (Table 2). Across species, there was a significant drought by origin interaction for plant size (Fig. 2a): While ancestral plants showed only a slight decrease of plant size in response to drought, the descendants strongly decreased plant size under drought. This observation is quite consistent across species, as there was no significant three-way interaction among treatment, origin and species in our model ($F_{8,329}$ = 0.45, p = 0.890).

Discussion

Seedling survival experiment

In our seedling survival experiment with four Mediterranean plant species, we showed that descendant seedlings produced less aboveground biomass but survived longer under drought than their ancestors. The four studied species varied in their survival ability and growth, and only for *A. maritima* smaller size was associated with longer survival. Similar results using a multi-species approach with ten grassland herbs were found by Harrison and LaForgia (2019). In their experiment, species with small seedlings and low specific leaf area had a higher survival ability under drought. A possible explanation for this is reduced evapotranspiration through a decreased leaf number, leaf size and branching, and lower plant biomass (Aroca 2012). These observations also correspond with the notion that plants in dry conditions often decrease aboveground biomass production and allocate more biomass to roots leading to a higher root-shoot ratio (Martin and Stephens 2006; Villagra and Cavagnaro 2006; Erice et al 2007). Of course, other traits can also be responsible for an increased seedling drought tolerance as root structures such as hypocotyl hairs (Aronne and De Micco 2004) or seed size (Fenner and Kitajima 1999). Furthermore, according to the predictions of climate change for the Mediterranean region, with decreased rainfall and more frequent droughts, high plasticity in seedling traits could also be advantageous (Gimeno et al 2009). For example, Padilla and Pugnaire (2007) showed that a rooting depth responding plastically to water availability could be more important than a constitutively higher root:shoot ratio. In this experiment it seems likely that the reduction in aboveground biomass for *A. maritima* is an adaptation (Erice et al 2010) to increased drought frequency in the Mediterranean region over the last decades (Met office 2011). For the three other studied species we could not observe the above mentioned pattern. Possible reasons for that could be that these species have not really evolved yet, the differences cannot be seen at seedling stage or these species established one of the other mentioned or even alternative strategies to cope with drought during seedling stage.

Altogether, we show that seedling survival under drought has evolved in the last 21-26 years in four Mediterranean plant species. As those changes have evolved in a very short time (supposedly less than 10 generations assuming cycles more than two years per generation), our study suggests high selection pressures under the increasingly heat-wave prone Mediterranean climate.

Watering response experiment

In our watering response experiment with nine species from temperate Europe we subjected adult plants to drought which generally led to decreased plant sizes and aboveground biomasses. We found no general cross-species differences in mean traits between descendants and ancestors, but there was a general difference between ancestors and descendants in the plasticity of plant size in response to drought, with a much stronger decrease of size in the descendant plants. Since plant biomass and number of leaves or shoots were not affected, this stronger shift in plant size under dry conditions could be accompanied by changes in leaf morphology such as leaf thickness or shape which are known to be highly plastic traits (Gianoli and Gonzáles-Teuber 2005; Lázaro-Nogal et al 2015). A reduction of leaf area accompanied by increasing leaf thickness and/or more pubescent leaves may reduce evapotranspiration (Gianoli and Gonzáles-Teuber 2005) and can therefore be a successful strategy under drought (La Riva et al 2016).

In general plant responses to drought are very complex as drought affects plants at various development stages and different tissues (Yordanov et al 2000). Our experiment does not allow us to identify the processes underlying the observed patterns which could include increased resource allocation to roots (Martin and Stephens 2006; Villagra and Cavagnaro 2006; Erice et al 2007), reduced evapotranspiration (Aroca

2012), reduced photosynthesis rate and increased oxidative stress (Zlatev and Lidon 2012) or a combination of these and other factors.

We also found larger plant size in the control treatment for the descendants compared to ancestors, which may be an adaptation of the species' life history. As flowering onset is often related to plant size (Vile et al 2006; Sun and Frelich 2011), we argue that plants grow and develop fast when water supply is satisfactory to avoid potential drought stress later on in their life cycle (Grene et al 2011).

In general higher environmental heterogeneity should favour greater phenotypic plasticity (Alpert and Simms 2002; Bradshaw and Holzapfel 2006; Matesanz et al 2010). In the regions of origin of the study species, drought frequency has increased over the last 20 years (Spinoni et al 2018), and environmental conditions thus became more unpredictable (Altvater et al 2011) and may thus have favoured evolution of plasticity. It is common that, as in our experiment, differentiation in phenotypic plasticity only occurs in some traits (Heschel et al 2002), and of course the mere observation of higher plasticity in a phenotypic trait does not necessarily mean that this is an adaptive change. Nevertheless, it is known that plasticity, like mean trait values, may evolve by natural selection (Ackerly et al 2000; Richards et al 2006). To test whether the observed higher plasticity in plant size of the descendant plants is an adaptive change to increased drought frequencies requires further experiments that include longer-term measurements of plant fitness (Richards et al 2006). Ideally these experiments would take place at the species' sites of origin and incorporate a large number of populations with different magnitudes of climate change during the past decades.

We acknowledge that in both experiments our results could have been influenced by unintentional selection during sampling (Hay and Smith 2003; Hoban and Schlarbaum 2014) or by potential selection during storage (Crawford et al 2007; Godefroid et al 2010). Furthermore, we could not rule out maternal effects, which could have been passed on over multiple generations (Gimeno et al 2009). However, we expect that random processes or unintentional selection are unlikely to have a stronger effect than those exerted by the drought treatments, as seedling recruitment and responses to drought are under high selection pressure (Schupp 1995; Fenner and Kitajima 1999). Moreover, the observed higher plasticity in descendants was consistent for seven out of the nine studied species (Fig. S1), which is highly unlikely if part or all of this effect was due to random processes or unintentional selection on each species separately.

Conclusions

Ongoing climate change is expected to influence the evolution of plant populations, but so far experimental tests of this are rare. Our multi-species experiments using species from two different biogeographic regions in Europe indicate that plants have indeed evolved within the last decades, possibly in response to increased drought frequencies. The observed evolutionary changes involve both changes in trait means and in plasticity in response to drought. Given the increased occurrence of drought events, our results suggest that climate change may have already influenced the evolutionary trajectory of plant species in different regions of Europe. Our study also demonstrates that the resurrection approach is a powerful tool for studying rapid evolutionary changes in plants. Future studies should corroborate our results across other species, ideally with multiple populations for each, using seeds from plants that underwent one generation in a common garden to exclude possible maternal effects. To disentangle adaptive from non-adaptive responses to recent climate change, these experiments should incorporate fitness measures and comparative transplantations of descendants and ancestors into their original habitat.

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Supplementary material

Fig. S1 Individual reaction norm plots of plant size for all tested species

Table S1 Raw data of the seedling survival experiment

Table S2 Raw data of the watering response experiment

Figures and tables

Figure captions

Fig. 1 Mean number of days until mortality after watering ceased (a) and aboveground biomass at harvest (b) of seedlings of four Mediterranean species (*Anthemis maritima, Matthiola tricuspidata, Medicago marina, Plantago subulata*) from two different temporal origins (ancestors vs. descendants). The bars show means and standard errors. * = $p<0.05$, *** = $p<0.001$

Fig. 2 Reaction norm plots of plant size (a), number of leaves or shoots (b) and aboveground biomass (c) in the watering response experiment. The data are always transformed and across all nine species from two different origins (ancestors vs. descendants). Error bars show standard errors.

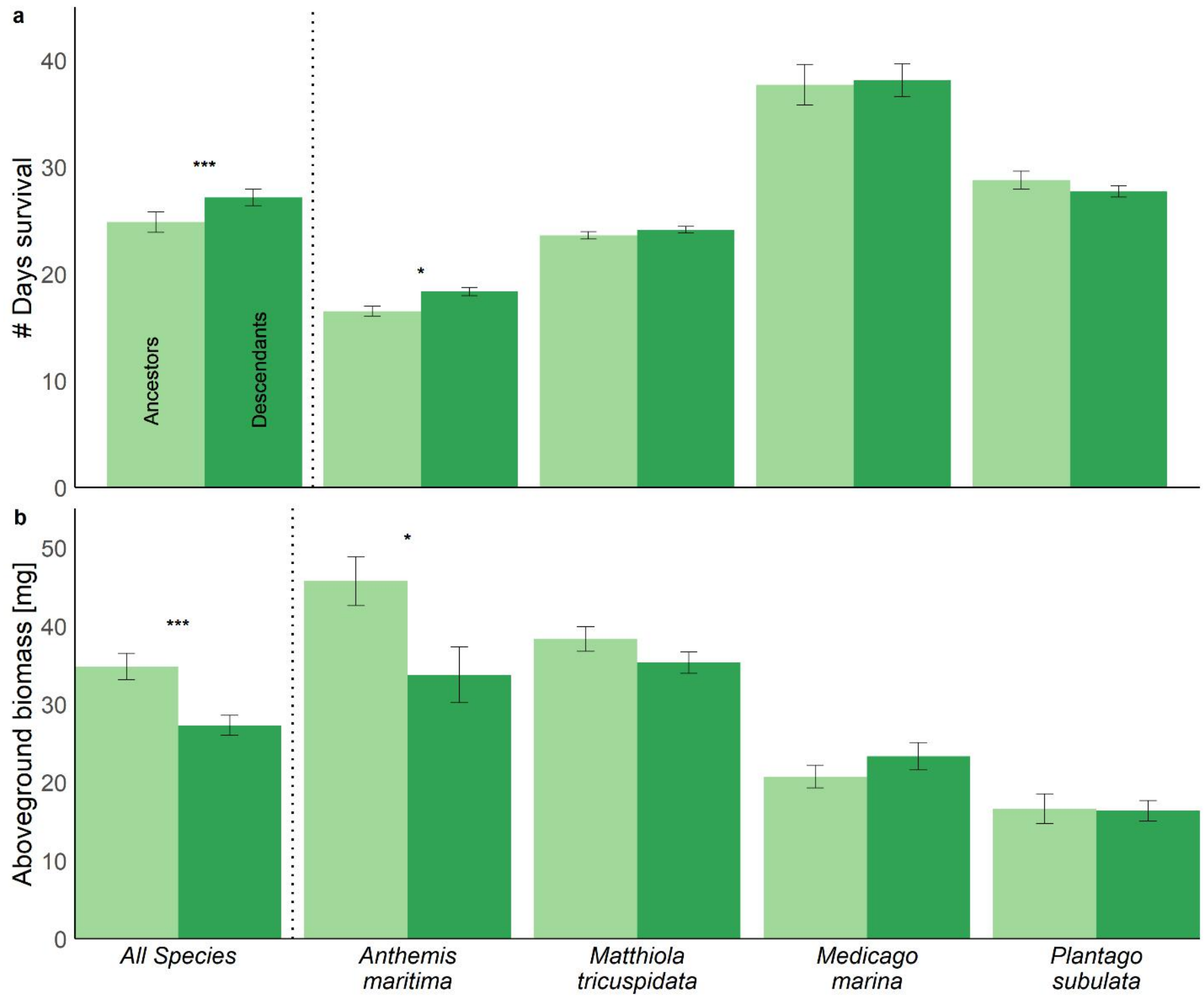
a
Days survival
0
10
20
30
40
Ancestors
Descendants
***
*
b
Aboveground biomass [mg]
0
10
20
30
40
50
***
*
All Species
Anthemis maritima
Matthiola tricuspidata
Medicago marina
Plantago subulata

Fig 1

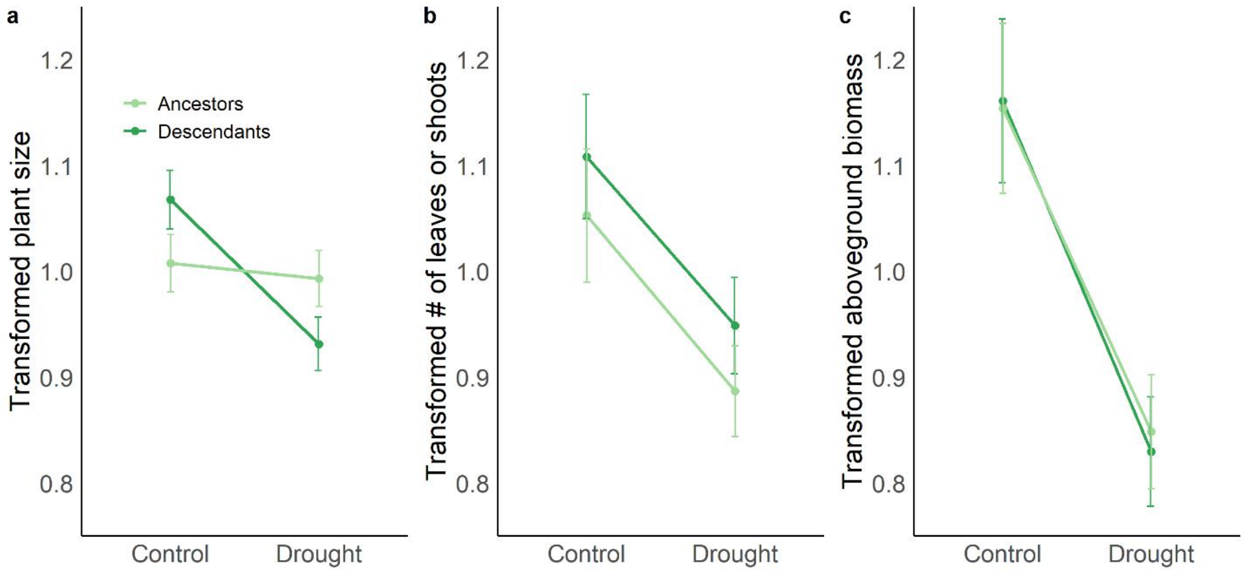
a
b
c
Transformed plant size
Transformed # of leaves or shoots
Transformed aboveground biomass
1.2
1.1
1.0
0.9
0.8
Ancestors
Descendants
Control
Drought

Fig 2

Table 1 Study species used in the two experiments with details on plant family, seedbank, country, year of collection, life form, measured traits in the watering response experiment and the number of replicates used.

Species	**Family**	**Seedbank**	**Country**	**Collection year**	**Measure of**		**Replicates**
					plant size	**Number of leaves of shoots**	
Seedling survival experiment: Mediterranean species							
Anthemis maritima	Asteraceae	CBNMed	France	1992	-	-	30
Matthiola tricuspidata	Brassicaceae	CBNMed	France	1994	-	-	30
Medicago marina	Fabaceae	CBNMed	France	1992	-	-	20
Plantago subulata	Plantaginaceae	CBNMed	France	1997	-	-	9
Watering response experiment: Temperate species							
Centaurium erythraea	Gentianaceae	Meise	Belgium	1992	diameter	leaves	12
Clinopodium vulgare	Lamiaceae	Meise	Belgium	1992	height	shoots	12
Dianthus carthusianorum	Caryophyllaceae	Osnabrück	Germany	1993	diameter	leaves	7
Digitalis lutea	Plantaginaceae	Meise	Belgium	1992	diameter	leaves	12
Leontodon hispidus	Asteraceae	Meise	Belgium	1995	diameter	leaves	12
Melica ciliata	Poaceae	Meise	Belgium	1992	height	shoots	7
Pimpinella saxifraga	Apiaceae	Meise	Belgium	1992	diameter	shoots	8
Sedum album	Crassulaceae	Meise	Belgium	1992	diameter	shoots	12
Teucrium chamaedrys	Lamiaceae	Meise	Belgium	1992	height	shoots	12

Table 2 F-values of linear model analyses of the watering response experiment, each testing for effects of treatment (T; drought vs. control), origin (O; ancestors vs. descendants), and their interaction (T x O). , and, for the all-species model, species as explanatory factors. The arrows indicate the direction of a significant effect (↓ = transformed values of the descendants or drought, respectively, are smaller). Shading indicates the range of the p-value (light gray: $p<0.1$, medium gray: $p<0.05$, dark gray: $p<0.001$). Degrees of freedom for all tested effects was 1 and varies for the residuals.

	Plant size			Number of leaves or shoots			Aboveground biomass		
	T	O	T x O	T	O	T x O	T	O	T x O
All Species	12.02↓	0.02	5.95	9.84↓	1.44	0.03	27.86↓	0.01	0.01
Centaurium erythraea	7.91↓	3.69	4.04	0.03	3.94	0.17	4.95↓	14.08↑	2.87
Clinopodium vulgare	0.28	0.05	0.68	0.25	0.73	0.52	2.03	0.07	0.38
Dianthus carthusianorum	0.25	0.11	0.53	1.78	0.20	1.31	0.03	8.79↓	3.08
Digitalis lutea	0.73	0.04	0.04	7.23↓	2.87	0.09	3.75	0.13	0.36
Leontodon hispidus	20.25↓	1.64	0.86	7.12↓	3.71	0.02	29.15↓	2.85	0.53
Melica ciliata	0.36	0.19	2.43	1.13	9.58↑	0.60	2.49	22.30↑	1.40
Pimpinella saxifraga	1.50	2.54	0.48	4.40↓	1.75	0.02	3.38	0.05	2.15
Sedum album	5.01 ↓	0.04	0.65	12.24↓	0.95	0.79	38.40↓	0.69	0.48
Teucrium chamaedrys	1.75	0.39	0.97	3.41	0.76	0.60	4.98↓	1.53	0.42