Historical comparisons show evolutionary changes in drought responses in European plant species after two decades of climate change

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Abstract

Plant populations must continuously adapt to ongoing global climate change, including warmer temperatures and more extreme weather events. One way to detect such evolutionary changes within plant populations is through historical comparisons where plants grown from seeds collected in the past (“ancestors”) are compared to freshly collected seeds from the same populations (“descendants”) in common garden experiments. Here, we used 21-26 year old seeds stored in seed banks for two multi-species experiments that investigated changes in phenotypic traits and their plasticity conferring drought tolerance in early life stages of European plant species from two biogeographic regions. In the first experiment we used seedlings of four Mediterranean species, ceased watering and recorded their day of mortality. In one of four species descendant seedlings survived significantly longer without watering and were smaller than the ancestral seedlings. In the second experiment we studied phenotypic responses to drought in juvenile plants of nine species originating from temperate regions in Europe. We found that descendant plants were generally taller under well-watered conditions but smaller under drought than their ancestors, thus showing stronger plasticity. Our historical comparisons thus suggest that some populations have likely evolved, through changes in trait means and plasticity, within the last decades, and in ways consistent with adaptation to increased drought. Using seed bank material for historical comparisons has several weaknesses, such as unknown sampling protocols or invisible fractions. However, we show how accurately sampled and stored seed bank collections can be used similar to the resurrection approach for investigating rapid evolutionary processes in plants under climate change, opening up a vast amount of available ancestral seed material worldwide for similar studies.

Introduction

Climate change has increased dramatically over the last several decades (IPCC 2018), and plant populations are already responding (Peñuelas and Filella 2001, Parmesan and Yohe 2003). Projections for Europe forecast that, during the 21st century, annual precipitation sums will further increase in the north and decrease in the south (IPCC 2013). For central and western Europe, precipitation is expected to increase in the winter and decrease in the summer (IPCC 2013), leading to more droughts in the growing season. Moreover, the 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 strong stressors for plants (Jaleel et al 2009; Fleta-Soriano and Munné-Bosch 2016), and many plant populations may not be adapted to these novel conditions (Anderson et al 2012; Shaw and Etterson 2012). To avoid extinction, some plant populations migrate to track suitable conditions whereas others may respond through phenotypic plasticity or adaptive evolution (Holt 1990; Hoffmann and Sgrò 2011). Such plastic or evolutionary adjustments could for instance involve reduced growth, to reduce evapotranspiration (Kusaka et al 2005, Borrell et al 2014), or increased root-shoot ratio (Sharp and LeNoble 2002; Aroca 2012) to promote water uptake. In environments that become generally drier, constitutive changes in such traits may be adaptive. However, drought events are often periodic, which would render the ability to change functional trait values through phenotypic plasticity 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 (Scheepens et al 2018). Still, studies on the effects of climate change on plant populations often only consider changes in mean climate conditions (Bertrand et al 2011), despite the strong evidence for increased climatic variability both among and within years (IPCC 2013, Gherardi and Sala 2018), specifically more heavy rain events followed by longer dry periods in many regions (Kharin et al 2007).

Phenotypic plasticity itself can also evolve and is thought to be selected for particularly in spatially or temporally variable environments (Ackerly et al 2000; Richards et al 2006). For example, Lázaro-Nogal et al (2015) showed in a common garden study with *Senna candolleana* that populations from environments with stronger interannual precipitation variation 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, 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 of plants. The fate of plant populations will thus depend on their ability to adapt to altered climatic variability and increased drought intensities through evolution of plasticity and/or constitutive adaptation to drought.

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 (Franks et al 2007; 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 and species responded evolutionarily in the past is extremely valuable for making predictions for future population and species responses to environmental change (Orsini et al 2013; Franks et al 2018).

An increasing number of studies have used this “resurrection approach” to examine rapid evolution to increased drought. Some of these studies convincingly showed that plants adapted their phenology towards an earlier flowering in order to avoid drought (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) the descendants were better adapted to drought and grew larger, whereas Vigouroux and colleagues (2011) found the opposite results in a study with *Pennisetum glaucum* where descendant populations that experienced drier climates during 27 years grew smaller. Thus, although species may vary in their evolutionary responses to drought, some traits show consistent evolution across species. Multi-species resurrection experiments can elucidate such commonalities, and therefore improve our ability to forecast future evolution under climate change.

Seedling establishment is a key process for population survival (Grubb 1977), and seedlings are especially susceptible to drought (Moles and Westoby 2004). Therefore, the drought resistance of seedlings should be under high selection pressure in increasingly dry and more variable environments (Schupp 1995; Fenner and Kitajima 1999). In 2019, Dickman and colleagues published another study with *Mimulus laciniatus* showing that contemporary populations which experienced droughts during the last years germinated earlier (Dickman et al 2019). However, studies on evolution of drought resistance in early life stages are generally still scarce.

Here, we investigated whether single populations of multiple plant species from Mediterranean and temperate regions of Europe have evolved their drought tolerance over the last decades in response to more frequent and longer drought events (Met Office 2011; DWD 2018; IRM 2020), examining particularly early life stages of plants. To investigate this, we conducted two complementary common garden experiments in which we applied drought treatments to plants raised from seeds stored for at least 21 years in three different seed banks (ancestors) and from seeds that we collected from the same populations in 2018 (descendants). As seedlings are generally very sensitive to dehydration, especially in environments with large fluctuations in water availability and high chance for drought events (Padilla and Pugnaire 2007), we used four herbaceous Mediterranean species in the first experiment to test whether seedlings of the descendants survived longer without watering than the seedlings of their ancestors (“seedling survival experiment”). In the second experiment (“watering response experiment”) we worked with juvenile (i.e. establishing, non-flowering) plants from nine temperate European species which experience the lowest precipitation during early growth between April and June (Camarillo-Naranjo et al 2019, Harris et al 2020). We subjected ancestors and descendants to well-watered vs. dry conditions and compared their growth responses within the first weeks after germination 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 from the seed bank at the Conservatoire Botanique National Méditerranéen de Porquerolles (CBNMed, Hyères, France). For the watering response experiment investigating juvenile plants, the seed material was provided by the seed banks of Meise Botanic Garden (Belgium) and the Botanical Garden of the University 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 21 years. We mostly 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 evolution. To reduce outcrossing with other populations, we specifically chose species with relatively isolated (but large) populations.

The resurrection approach has strict criteria for seed sampling, with at least two time points for sampling, each time collecting >30 plants while keeping maternal lines separated (Franks et al. 2018). These criteria safeguard that genetic diversity within a population is captured sufficiently and that the original genetic structure is kept largely intact. As the materials from the seed banks were not originally collected with the aim to conduct resurrection experiments (e.g. the number of sampled individuals is often unknown, and all sampled seeds were bulked) our study does not fulfil these strict criteria. However, with two types of further information, we are convinced that seed bank material can be used in a similar way to the resurrection approach, and that historical comparisons based on it are meaningful.

The first type of evidence is information on sampling. All species occur rather abundantly in their original habitat, the amount of seeds within the stored lots was high (Table 1) and the collectors tried to maximize the number of sampled individuals. Thus, we are confident that the genetic diversity of seed bank collections we used is representative of what was present at the time of sampling.

The second important information comes from a molecular analysis using ddRAD-SNP marker data for all species comparing the relative genomic relatedness of ancestors to that of descendants. We show that the relatedness of plants is similar within ancestors and descendants for 12 out of the 13 species (Supplemental Information S1, Durka et al unpublished data), providing further support for similar sampling procedures and that sufficient seeds were sampled during both periods avoiding biased sampling of particular mother plants.

For the seedling survival experiment we used seeds of four Mediterranean species (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. Data from the Climatic Research Unit show that average temperatures between March and July have increased by 1.1°C and precipitation anomalies summed to a decrease of around 1.5mm per year during the last 30 years in comparison to 1900-1999 and precipitation variability (CV) during 2009-2018 was 35% larger than during 1988-1997 (Camarillo-Naranjo et al 2019, Harris et al 2020). After the collection of ancestor seeds, 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-desiccated and stored at 17°C at the CBNMed until November 2018.

For the watering response experiment, we used seeds of nine temperate species (Table 1): *Centaurium erythraea*, *Clinopodium vulgare*, *Dianthus carthusianorum*, *Digitalis lutea*, *Leontodon hispidus*, *Melica ciliata*, *Pimpinella saxifraga*, *Sedum album* and *Teucrium chamaedrys*. The seeds of these nine species had been collected between 1992 and 1995 in Belgium (two different regions) and close to Osnabrück (Germany). Comparing the last 30 years with 1900-1999, the average temperatures between March and July have increased by 1.1°C for *D. carthusianorum* close to Osnabrück*,* also by 1.1°C for *L. hispidus* and by 0.9°C for the remaining species in Belgium (Camarillo-Naranjo et al 2019, Harris et al 2020). During the 30-year period, precipitation in spring and summer decreased by about 29mm per year in all three regions but precipitation variability (CV) differed in comparison of 2009-2018 to 1988-1997, with an 45% decrease for *D. carthusianorum*, no change for *L. hispidus* and a 25% increase for the remaining species in Belgium (Camarillo-Naranjo et al 2019, Harris et al 2020). After ancestor seed collection, all seeds had been cleaned, dried at 15% relative humidity and then stored at -20°C at Meise Botanic Garden and the Botanical Garden of the University of Osnabrück until we received the seed materials in November 2018.

To obtain the descendants, seeds of all species were collected from the exact same populations in spring (Mediterranean species) and summer (temperate species) of 2018. In each population, we sampled between 15 and 103 individuals

(Table 1) and then bulked all seeds to have a comparable seed mix as for the ancestors.

Seedling survival experiment

For the seedling survival experiment with the Mediterranean plants, we initially sowed 100 seeds per temporal origin (i.e. ancestors and descendants) of each species. To break physical seed dormancy of *M. marina*, we scarified the seeds of this species by softly scrubbing them with sandpaper (Royal Botanic Gardens Kew 2020), and to reduce the growth of microbes during germination, we surface-sterilized all seeds for ten minutes 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 50% relative humidity) with a light/dark cycle of 8/16 hours and temperatures of 23/18 °C and recorded germination success every second day. The germination rates were similar for ancestor and descendant seeds of *A. maritima* and *M. tricuspidata* but they differed for *M. marina* and *P. subulata* (Table 1).

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 (see Table 1 for the numbers of individuals) into every other cell so that the seedlings did not grow directly next to each other. We planted the ancestors and descendants in an alternating pattern. To identify the seedlings, we noted their positions but did not use any labels 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 µmol·m^{-2}·s^{-1}, 50% relative humidity). The seedlings were watered regularly for 2-3 weeks (depending on the species) to allow their establishment. After that, we stopped watering to simulate 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 72 h, and weighed it.

We used linear models to analyse how the temporal origin (ancestors vs. descendants) of seedlings affected their numbers of days of survival (i.e. time between start of the drought treatment and death) and aboveground biomass. To account for species differences, we included species identity in the model as a fixed factor. For the analysis of numbers of days of survival we additionally included the numbers of days between transplanting and start of the experiment as a covariate, and for the analyses of aboveground biomass we included the total lifespan of the seedling as a covariate. In addition to the full multi-species model we also analysed the data separately for each species, using the same models but excluding species identity. Finally, we used linear models to test whether the aboveground biomass of a plant predicted its number of days of survival, while correcting for the total lifespan of the seedling by including it as a covariate. For all models we visually checked the normality of residuals and heteroscedasticity. All analyses were done in R (Version 4.0.2) using the package *plyr* (Wickham 2011). The data are accessible as supplementin Table S1.

Watering response experiment

For the watering response experiment with temperate plant species, we germinated 100-1000 seeds per temporal origin (see Table 1 for precise numbers) in trays filled with a standard potting soil (Einheitserde®, BioLine, Pikiersubstrat), with a separate tray for each origin. 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 under a natural spring daylight regime. We kept the seedlings in these trays for three months before the start of the experiment

For the main experiment, we filled 9 × 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). In early June 2019, we transplanted all seedlings into the pots always with 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) as well as, depending on the species, the numbers of leaves or shoots (Table 1). After two weeks, we split all juvenile plants into a well-watered control group and a drought group, with 7-12 replicates per temporal origin 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. We re-randomized all pots in the greenhouse weekly. After eight weeks we repeated the growth trait measurements and then harvested all plants and determined their aboveground biomass after oven-drying at 60 °C for three days.

For the data analyses we square-root-transformed the number of leaves and aboveground biomass. In order to be able to compare different measurements across the nine species, we standardised all data to a mean of 0 and a standard deviation of 1. We then analysed the variation in plant size, number of leaves or shoots, and aboveground biomass with linear models that included temporal origin (ancestors vs. descendants), treatment (drought vs. control) and species, and all possible interactions, as fixed explanatory variables. A two-way interaction between temporal origin and treatment would suggest that plants have evolved a different response to drought, and a three-way interaction between temporal origin, treatment and species would suggest that species vary in their evolutionary responses to drought. In addition to the multi-species analyses, we also analysed the data for each species separately, using linear models that included only temporal origin, treatment and their interaction. As the sizes of transplanted seedling differed, we corrected for this by including the initial size measurements 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 (Version 4.0.2) using the package *plyr* (Wickham 2011). Data is accessible as supplementin Table S2.

Results

Seedling survival experiment

Across species, 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 temporal origin (ancestors vs. descendants;

Fig. 1a, $F_{3,208} = 2.74$, p = 0.04). The overall effect of temporal origin was driven by one of the species, *A. maritima*, since only descendants of this species showed a significantly longer survival than their ancestors in the individual-species analyses (Fig. 1a, $F_{1,60} = 6.01$, p = 0.017). Across species, 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 temporal 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 temporal 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 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

The drought treatment had a significant effect on all three measured growth traits. Across all nine species, plants grown under drought conditions were smaller, produced fewer branches or leaves and had a lower aboveground biomass (Fig. 2a-c, Table 2). These observations were also consistent at the species level: in all species where a significant effect occurred, drought decreased plant growth (Table 2). Seven out of the nine tested species were affected in at least one of the measured traits. There were no significant interactions between species and treatment. The temporal origin did not affect plant size in any of the studied species, but we found a significant difference in the number of leaves or shoots and in aboveground biomass between the ancestors and descendants of two and three species, respectively. In *C. erythraea* and *M. ciliata,* descendants produced significantly more leaves or shoots and biomass, but in *D. carthusianorum* descendants produced less biomass (Table 2). Across species, there was a significant drought-by-temporal origin interaction for plant size (Fig. 2a, Table 2). 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 consistent across species, as there was no significant three-way interaction among the watering treatment, temporal origin and species in our model ($F_{8,329}$ = 0.48, p = 0.87). However, none of the individual-species models showed a significant treatment by temporal origin interaction for plant size (Table 2).

Discussion

To test for recent evolutionary responses of plants to climate change, we compared the drought sensitivity of ancestral and descendent plants of several Mediterranean and temperate plant species. Similar to the so-called resurrection approach, we used seed materials from seed banks, together with seeds re-collected from the same populations several decades later. In two experiments we focused in particular on the drought sensitivity of early plant life-stages.

Seedling survival experiment

In our seedling survival experiment with Mediterranean plant species, we found that in one of the four studied species, *Anthemis martima*, descendant seedlings survived longer under drought than their ancestors and produced less aboveground biomass. Although our data cannot be used to prove that the observed evolutionary changes are adaptive, our observations are consistent with what would be expected if adaptation to drought had occurred in the studied population during the last decades.

Survival under drought can be enhanced by a small plant size, as we observed for *A. maritima*. In a multi-species approach Harrison and LaForgia (2019) compared seedling survival of ten grassland herbs under different water availabilities. They showed that the survival rate of smaller seedlings was higher under dry conditions. A possible explanation for this is a reduced evapotranspiration through decreased leaf number, leaf size and branching and lower plant biomass (Aroca 2012). These observations also fit to the observation 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). However, increased seedling drought tolerance can also be mediated by other traits such as root structures (e.g. hypocotyl hairs; Aronne and De Micco 2004), or seed size (Fenner and Kitajima 1999). However, it is also possible that the observed reduction in plant size was the result of passive stress responses instead of above-mentioned active responses of plants to droughts.

Our main research question was to test for evolutionary changes between ancestors and descendants, and our historical comparison, a somewhat less strict version of the resurrection approach, has some weaknesses here, particularly when interpreting biomass results. First, as we did not grow a ‘refresher generation’ of ancestors and descendants prior to our main experiment, we cannot exclude potential storage or maternal effects (Franks et al 2018). Second, if stored seeds have low germination rates, there is a possibility of “invisible fractions” (Weis 2018), with germinating individuals representing only a subset of the stored phenotypes. In *A. martitima* the germination rate for the ancestors was only 45%, so we cannot rule out such invisible fraction effects. A third potential drawback of such historical comparisons with seed bank material not designed for these purposes is that sampling efforts can be very different for seeds from different periods. Luckily, our molecular analysis found similar levels of relatedness among ancestors and descendants, indicating that the sampling probably has been conducted in a similar way and that sampling effort was sufficiently high.

While descendants of *A. maritima* showed improved drought resistance compared to their ancestors, we did not find similar patterns for three other species. Possible reasons for this could be that these species have not evolved due to lack of genetic variation or other evolutionary constraints (e.g. trade-offs) preventing evolution in specific phenotypes. Alternatively, it is also possible that these species evolved different (phenological) strategies to cope with drought during the seedling stage which we did not explicitly study.

In summary, we show that seedling survival under drought has likely evolved in the last decades through adjustments of phenology and growth strategy in one out of four studied Mediterranean plant species. To confirm the adaptive significance of the observed differences, and to disentangle evolutionary processes from other potential influences such as maternal or storage effects, more detailed experiments, including an additional refresher generation, are necessary (Franks et al 2018).

Watering response experiment

In our watering response experiment with nine species from temperate Europe, we subjected juvenile plants to drought that generally led to decreased plant sizes and aboveground biomass. Across species, we found no differences in mean traits between ancestors and descendants, but there was an overall 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 precipitation variability has increased for most of the studied species during the last decades this observation is in line with predictions that such conditions favour the evolution of increased phenotypic plasticity (Sultan and Spencer 2002, Alpert and Simms 2002, Gianoli and Valladares 2012).

However, we did find an interaction between treatment and temporal origin for plant size in none of the single-species analyses, which is probably partly explained by the moderate replicate numbers per species. Nevertheless, seven out of nine species showed a trend in this direction (Fig. S2), and the three-way-interaction with species, temporal origin and treatment was insignificant indicating similar cross-species patterns. Since plant biomass and number of leaves or shoots were unaffected, this stronger shift in plant size under dry conditions could be accompanied by other functional traits we did not measure in our study such as leaf thickness or leaf shape which are known to be highly plastic (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).

Plant responses to drought are generally complex, as drought affects plants at various developmental stages and in different tissues (Yordanov et al 2000). Our experiment does not allow us to – but future studies should – identify the processes underlying the observed patterns that may 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 significantly larger plant sizes but not higher aboveground biomasses in the control treatment for the descendants compared to ancestors across species. This may be an adaptation of the species' life cycles: 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 sufficient to escape potential drought stress later in their life cycle (Grene et al 2011). When interpreting the results of our study we should keep in mind though that our drought treatment was simplified, with water applied at constant low versus constant normal levels. In nature, patterns of water availability may be more variable, and we do not know how our plants would e.g. have responded to drought after a period of sufficient watering. This is important given that under ongoing climate change not only mean precipitation but also temporal patterns are changing.

Greater environmental heterogeneity in space or time, when perceived within the organism’s – or its immediate descendants’ – lifetime, is generally expected to favour greater phenotypic plasticity (Alpert and Simms 2002; Bradshaw and Holzapfel 2006; Matesanz et al 2010). In the regions of origin of most of the study species, drought frequency has increased over the last 20 years (Spinoni et al 2018), and environmental conditions have thus became more unpredictable (Altvater et al 2011). This could have favoured evolution of stronger plasticity through natural selection for more plastic genotypes (Ackerly et al 2000; Richards et al 2006). To test whether the observed greater plasticity in plant size of the descendant plants is an adaptive change requires further experiments that include longer-term measurements of plant fitness (Richards et al 2006). Ideally such experiments should take place at the species’ sites of origin and incorporate a large number of populations which experienced different rates of climate change, and in particular increased precipitation variability, during the past decades.

Although our results may be influenced by other factors, we are confident that we observed a true evolutionary pattern here that is common in nature: greater plasticity of descendants – as a trend – was consistent for seven out of the nine studied species (Fig. S2), which is very unlikely if part or all of these patterns were due to chance or unintentional selection during sampling and storage or due to maternal effects on each species separately (see first discussion section above). Furthermore, germination rates in most species were high, and there were no relationships between germination rate and plasticity, suggesting that variation in germination rates did not affect other traits. However, we cannot completely exclude potential storage effects or hidden fractions, especially for *L. hispidus* where germination rates differed strongly between ancestors and descendants (Table 2).

Random processes or unintentional selection are unlikely to have stronger effects than those exerted by the drought treatments, which pose strong selection pressures on seedling recruitment and drought responses (Schupp 1995; Fenner and Kitajima 1999).

Using seed bank material for historical comparisons

Resurrection studies are a powerful tool for studying recent evolution, but the appropriate genetic resources are rarely available. Large-scale long-term efforts have recently been set up to conduct powerful resurrection studies in the future (e.g. *Project Baseline*, Etterson et al 2016). However, if material from regular seed banks could be used for similar before-after comparisons, it would open up a vast resource for environmental change research. Although seed banks often lack population replicates within species, multi-species approaches can make studies more powerful by testing for common evolutionary patterns across taxa.

Despite previously mentioned shortcomings of our study, we show that it is possible to use seed bank material, not explicitly collected for resurrection studies, for similar historical comparisons. In our study genomic relatedness analyses indicated that the ancestor and descendant seed pools were similar, and that seed sampling has been conducted in a comparable way. Our molecular data also suggests that the genetic diversity of seed bank collections and newly collected seeds was sufficiently large for conducting the experiments. We are therefore reasonably confident that the use of seed bank material in our study was meaningful. Our approach opens up a new avenue for studies on recent plant evolution and may be a useful complement to other approaches that study contemporary populations or use other stored materials such as herbarium specimen (Lang et al 2019, DeLeo et al 2019).

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 historical comparisons using taxa from two different biogeographic regions in Europe indicate that plants have evolved within the last decades, possibly in response to increased drought frequencies. We observed evolutionary changes in several, but not all, species, in both trait means and trait plasticity in response to experimental drought. Given the increased occurrence of drought events in most of the populations of origin, our results suggest that climate change may have already influenced the evolutionary trajectory of many plant species in different regions of Europe. Our study also demonstrates that historical comparisons similar to the resurrection approach can be made using plants from seed bank collections, and are a powerful tool for studying rapid evolution in plants. There is great potential for future studies to use of the wealth of seed bank collections for investigating rapid adaptation to recent environmental changes. Replicated populations of the same species may be scarce in seed banks, which is why a multi-species approach is generally advantageous. Ideally, seeds from a refresher generation should be used to minimize possible maternal effects. To disentangle adaptive from non-adaptive and maladaptive responses to recent climate change, future experiments should incorporate fitness measures and comparative transplantations of descendants and ancestors into their original habitat.

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Supplementary material

S1 (including Fig. S1) Comparing genomic relatedness and diversity between ancestral and descendant populations; ddRAD library preparation, SNP genotyping and population genomic analyses

Fig. S2 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 of survival 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 transformed and averaged across all nine species from two temporal origins (ancestors vs. descendants). Error bars show standard errors.

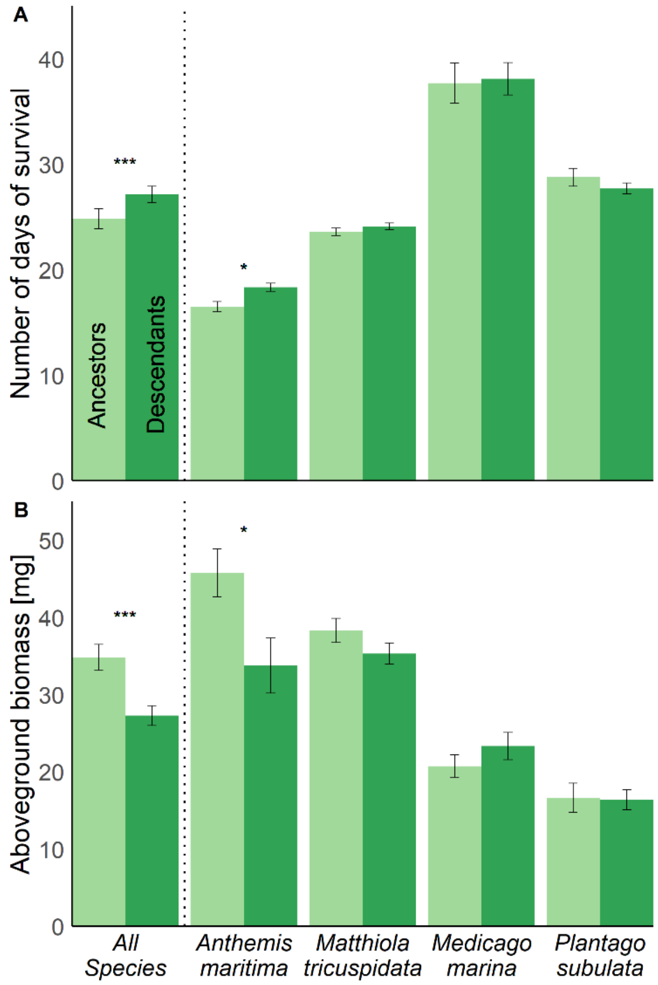

Fig 1

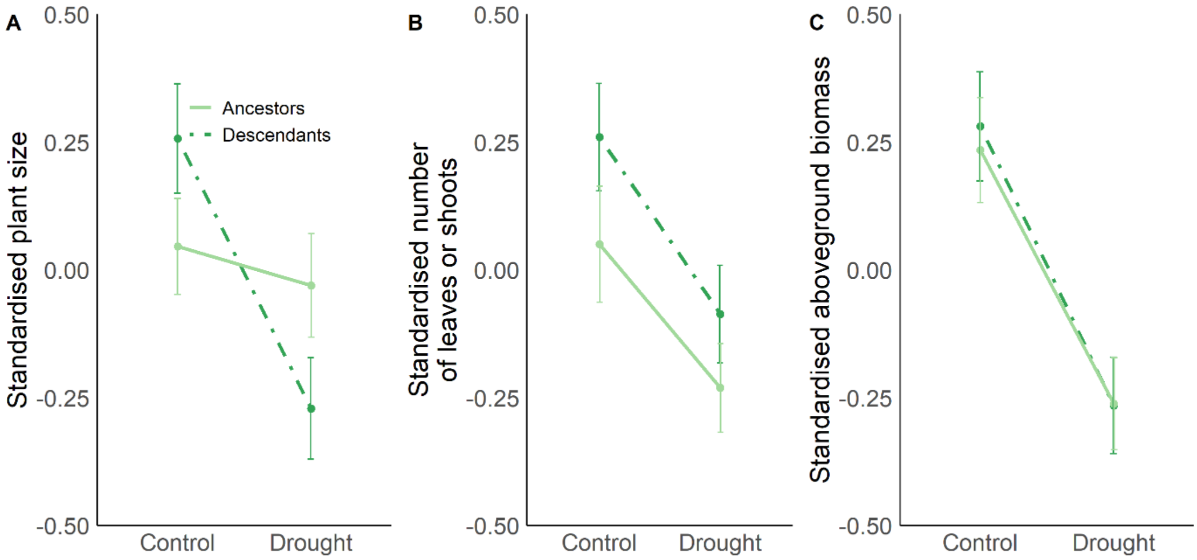
A
B
C
Standardised plant size
Standardised number of leaves or shoots
Standardised aboveground biomass
0.50
0.25
0.00
-0.25
-0.50
Ancestors
Descendants
Control
Drought

Fig 2

Table 1 Study species used in the two experiments with details on plant family, seedbank, year of collection, amount of stored seeds in the seedbanks, number of sampled individuals (2018), measured traits in the watering response experiment, the number of seeds used, germination rates and number of replicates for each treatment and temporal origin within the experiments.

Species	**Family**	**Seedbank**	**Collection year**	**Amount of stored seeds**	**Number of sampled individuals in 2018**	**Measure of**		**Number of used seeds and germination rate**		**Replicates**
						plant size	**Number of leaves of shoots**	ancestors	descendants	
Seedling survival experiment: Mediterranean species										
Anthemis maritima	Asteraceae	CBNMed	1992	1000	80	-	-	100 (25%)	100 (33%)	30
Matthiola tricuspidata	Brassicaceae	CBNMed	1994	1000	15	-	-	100 (95%)	100 (98%)	30
Medicago marina	Fabaceae	CBNMed	1992	500	50	-	-	100 (45%)	100 (90%)	20
Plantago subulata	Plantaginaceae	CBNMed	1997	500	103	-	-	100 (78%)	100 (29%)	9

Watering response experiment: Temperate species										
Centaurium erythraea	Gentianaceae	Meise	1992	1000	20	diameter	leaves	200 (50%)	1000 (50%)	12
Clinopodium vulgare	Lamiaceae	Meise	1992	1000	47	height	shoots	200 (75%)	200 (90%)	12
Dianthus carthusianorum	Caryophyllaceae	Osnabrück	1993	500	20	diameter	leaves	100 (25%)	100 (50%)	7
Digitalis lutea	Plantaginaceae	Meise	1992	2500	20	diameter	leaves	500 (20%)	500 (30%)	12
Leontodon hispidus	Asteraceae	Meise	1995	1000	20	diameter	leaves	300 (30%)	300 (80%)	12
Melica ciliata	Poaceae	Meise	1992	1000	21	height	shoots	200 (75%)	150 (50%)	7
Pimpinella saxifraga	Apiaceae	Meise	1992	1000	20	diameter	shoots	200 (50%)	200 (25%)	8

Sedum album	Crassulaceae	Meise	1992	1000	20	diameter	shoots	500 (20%)	500 (20%)	12
Teucrium chamaedrys	Lamiaceae	Meise	1992	1300	20	height	shoots	200 (20%)	300 (20%)	12

Table 2 F-values of linear model analyses of the watering response experiment, each testing for effects of treatment (T; drought vs. control), temporal origin (O; ancestors vs. descendants), and their interaction (T × O), and, for the cross-species model, species as explanatory factors (results not shown). The arrows indicate the direction of a significant effect (↓ / ↑ = transformed values of the descendants or drought, respectively, are smaller/larger). Shading indicates the range of the p-value (light 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 × O	T	O	T × O	T	O	T × O
Cross-species model	14.88 ↓	<0.01	6.16	10.56 ↓	3.60	0.05	41.90 ↓	0.25	0.01
Centaurium erythraea	7.91 ↓	3.69	4.04	0.01	4.22 ↑	0.17	5.50 ↓	15.40 ↑	2.87
Clinopodium vulgare	0.28	0.05	0.67	1.07	0.62	0.61	0.65	0.09	0.67
Dianthus carthusianorum	0.25	0.11	0.47	1.19	0.51	0.51	0.07	10.23 ↓	4.92
Digitalis lutea	0.73	0.04	0.04	7.38 ↓	2.74	0.39	5.12↓	<0.01	0.21

Leontodon hispidus	20.25 ↓	1.64	0.86	7.26 ↓	3.13	<0.01	29.44 ↓	3.86	0.81
Melica ciliata	0.36	0.19	2.43	1.55	9.58 ↑	0.56	2.77	23.24 ↑	0.60
Pimpinella saxifraga	1.50	2.54	0.48	5.54 ↓	1.55	0.10	4.12	0.16	2.24
Sedum album	5.01 ↓	0.04	0.65	12.23 ↓	0.61	0.90	37.07 ↓	0.23	0.73
Teucrium chamaedrys	1.75	0.39	0.97	1.34	1.62	0.92	3.02	2.63	0.88