Transgenerational responses to climate change in Mediterranean annual species with contrasting functional strategies

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A B S T R A C T

Increasing temperature and aridity predicted by climate change models in the Mediterranean region will pose a significant challenge for the survival of some plant species. The mechanisms by which species modify their phenotype across generations in response to the environment have been identified as essential to cope with the new climatic conditions. However, this transgenerational transfer remains unexplored for species with contrasted resource-use strategies (fast-growing or acquisitive versus stress-tolerant or conservative species). For this purpose, transgenerational responses to warming and drought of annual plant species with different resource-use strategies were assessed by conducting three experiments. In a field experiment, climatic treatments of increased temperature and rainfall exclusion were imposed on five herbaceous species typical of Mediterranean grasslands. The descendants of the individuals sampled in the field were employed in two greenhouse experiments to: (1) analyze the transgenerational response by re-imposing warming and drought conditions, and (2) identify possible maternal effects by maintaining them at optimal conditions of air temperature and water availability and comparing with maternal performance. We measured reproductive output, plant growth and survival variables as plant performance indicators. We found contrasting transgenerational responses to warming and drought depending on the functional strategy of the species. Direct effects of the abiotic stressors on parental plants were retained or new ones appeared in their offspring, with special negative impact on one of the most acquisitive species (*Crepis capillaris*). In contrast, the most conservative species (*Bromus madritensis*) highly tolerated increased climatic stress but deleterious maternal effects were registered, given the high negative impact on the performance of the descendants of maternal plants that were subjected to climatic treatments. Our study highlights the importance of evaluating plant responses to stress across generations to better understand their potential ability to face new environmental conditions in species with different functional strategies.

1. Introduction

Phenotypic plasticity, both within and across generations, is a mechanism by which species can adjust their phenotype to rapid environmental changes (Fuxjäger et al., 2019; Matesanz and Ramírez-Valiente, 2019) without alterations of DNA sequence (Puy et al., 2021). In particular, plasticity across generations occurs when parental environment determines the response of the progeny in future environments, being the influence of the maternal plant (maternal environmental effects) a specific and frequently studied form of this plasticity (Agrawal, 2002; Marshall, 2008). At the beginning, plasticity across generations was understood simply as a consequence of the

negative effects that stressful conditions of the parents induced in their offspring (Falconer, 1983). However, recent studies have assessed that its contribution to plant performance may be adaptive if the influence of the ancestor to the progeny improve its fitness regarding to the parental performance (Colicchio and Herman, 2020; Galloway and Etterson, 2007; Herman and Sultan, 2011; Mousseau and Fox, 1998). Regarding the mechanisms underlying this type of non-genetic inheritance, some studies point to changes in the nutritional status and composition of the seeds (Van Dam and Baldwin, 2001) and, more recently, to epigenetic changes (Weinhold, 2018). It has also been found that annual plant species are generally more susceptible to show transgenerational responses than perennials (Yin et al., 2019), making these species suitable

for use as study models. At present, many aspects as the environmental cues that promote transgenerational responses, their evolutionary role or their study in different functional types of plants remain largely understudied (Donelson et al., 2018).

Plasticity across generations is expected to play an essential role in the response of plants to the current phenomena of climate change (reviewed in Donelson et al., 2018). The maintenance of the species under new climatic conditions will depend on their capacity for migration, adaptation and / or phenotypic plasticity (Hoffmann and Sgrò, 2011). However, changes in climate are expected to be so rapid that species will have to respond adaptively within a few generations to ensure their persistence (Kremer et al., 2012; Shaw and Etterson, 2012) since migration will not probably be able to keep pace with these changes (Loarie et al., 2009). In the new climatic scenarios, species are affected not only by the isolated effects of different abiotic drivers (e. g. warming or drought) but also by the interaction between these factors. Although these effects are global, they are uneven in magnitude depending on the ecosystem type and the region of the planet in which they occur (Walther et al., 2002). Particularly, the Mediterranean region has been identified as one of the main climate change hot-spots (Giorgi, 2006), and its ecosystems have been considered especially vulnerable due to a growing problem of desertification promoted by the increment in temperature and drought (Thuiller, 2007). For this reason, a better understanding of plant ability to cope with these ongoing abiotic stressors across generations is particularly necessary in Mediterranean-type ecosystems. The generation of this knowledge will help to establish the scientific basis for management plans aimed at mitigating potential effects of climate change on plant community dynamics and ecosystem functioning.

Plant species can respond to new climatic conditions through changes in their phenology, reproduction, growth and/or physiology (Gallego-Tévar et al., 2019; Gray and Brady, 2016; Pérez-Ramos et al., 2019; Qaderi et al., 2006), with potential repercussions on survival and fitness of the individuals (Anderson, 2016; Urban, 2015) and, ultimately, on plant community composition (Bellard et al., 2012). However, not all plant species react in the same way to climatic constraints. Species with contrasting resource-use strategies may respond differently to these abiotic stressors (Pérez-Ramos et al., 2020). The main resource-use strategies in plants are defined along the so-called leaf economics spectrum as a gradient of variation of some key foliar traits (Wright et al., 2004) that reveals the way in which abiotic resources are acquired and largely determines plant growth-form. At one extreme of the gradient are the *acquisitive species*, characterized by presenting thin leaves with high N content, a rapid uptake of resources and high growth rate, which are frequent in moist and nutrient-rich soils. At the opposite extreme are the *conservative species*, defined by showing high-density, low-N leaves, which are more common in low-productivity environments (Díaz et al., 2016, 2004; Poorter and Garnier, 1999; Reich, 2014; Wigley et al., 2016). It has been broadly demonstrated that plant species with more acquisition-related traits are particularly sensitive to stressful conditions. However, these species present a more efficient use of resources than species with resource-conservation traits when conditions are not limiting (Carvajal et al., 2017; Pérez-Ramos et al., 2013; Zhang et al., 2020). Regarding their transgenerational response, there are very few studies that have analyzed this aspect in relation to the resource-use plant strategy, giving rise to contradictory results. Thus, some authors have found that plants growing under stressful environments (e. g. recurrent droughts or high competition) induced the development of more resource-acquisition phenotypes in their offspring (Cerda, 2020), while other studies registered more conservative traits in the descendants (Carbone and Aguilar, 2021; Puy et al., 2021). However, to our knowledge, there are no studies comparing transgenerational responses of species with inherent contrasting resource-use strategies so it is not known whether these species will respond differently in their transgenerational adaptation, determining in a different way the long-term performance of their offspring.

Taking all the above into consideration, we conducted three studies, including field and greenhouse experiments, to analyze the effects of the two main drivers of climate change (warming and drought) across generations in dominant annual species with contrasting functional strategies in Mediterranean grasslands. Specifically, the questions we sought to answer were:

(i) Are warming and drought-induced effects on performance of acquisitive species quantitatively higher than those on conservative ones?

(ii) Do species with different resource-use strategies show different transgenerational responses to contrasting climatic conditions?

(iii) Does phenotypic variability in the response of the maternal plants influence the phenotype of their offspring depending on the resource-use strategy of the species?

This will allow us to deepen in the understanding of the contribution that non-genetic inheritance has on the responses to climate change in different functional groups of plant species. Likewise, the results of this study are expected to provide valuable information to anticipate the responses of plant species to climate change and develop management strategies accordingly.

2. Materials and methods

2.1. Species selection

Five species of abundant annual plants in Mediterranean grasslands were selected including the most representative families (Asteraceae, Geraniaceae, Poaceae and Plantaginaceae) of the herbaceous layer in these ecosystems. The selected species cover a wide range of the acquisition-conservation axis of plant ecological strategies (Gorné et al., 2020), from species with low-dense and N-rich leaves [*Crepis capillaris* (L.) Wallr (Asteraceae) and *Erodium moschatum* (L.) L'Hér. (Geraniaceae)] associated to a resource-acquisition strategy, to species with opposite trait values [*Bromus madritensis* L. (Poaceace)*, Anthemis arvensis* L. (Asteraceae) and *Plantago coronopus* L. (Plantaginaceae)] related to a resource-conservation strategy (Fig. 1, Fig. S1). We characterized the functional strategy of the different species by measuring four traits (leaf dry matter content, LDMC; specific leaf area, SLA; leaf size and foliar N content; see details of the methods in the last paragraph of this section) that are widely used as key descriptors of the leaf economics spectrum (Díaz et al., 2016; Reich, 2014; Wright et al., 2004).

2.2. Field experiment

In September 2016, six experimental plots of 4 × 6 m were randomly established in each of the three Mediterranean grasslands that were used as study sites ("La Morra"; 38°20'59.1"N 4°49'13.3"W, "Fuente La Raya"; 38°22'45.7"N 4°45'31.7"W and "La Panadera"; 38°22'58.4"N 4°45'29.3"W). These grasslands were very close each other (all of them located in "Los Pedroches Valley", Córdoba, Spain), and they were selected for this study due to their high similarity in climatic and edaphic conditions, as well as in geographical orientation and slope. The area is under Mediterranean climate, with an average annual temperature of 15.7ºC, an average annual precipitation of 550 mm (Escribano and Hernández, 2013) with 75–85 days of rain per year and 4–5 months of dry period in summer (AEMET, 2020; CLIMA, 2018). Vegetation is characterized by an abundant and mostly annual herbaceous layer (> 65% cover). The tree stratum is represented by scattered *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. individuals (ca. 20% cover).

An experiment with a factorial design was established at each of the 18 experimental plots in order to assess the effect of increasing temperature (warming, 'W' hereafter) and reduced precipitation (drought, 'D' hereafter) on the selected species that naturally occurring at the three grasslands. To simulate warming conditions, we used hexagonal open-
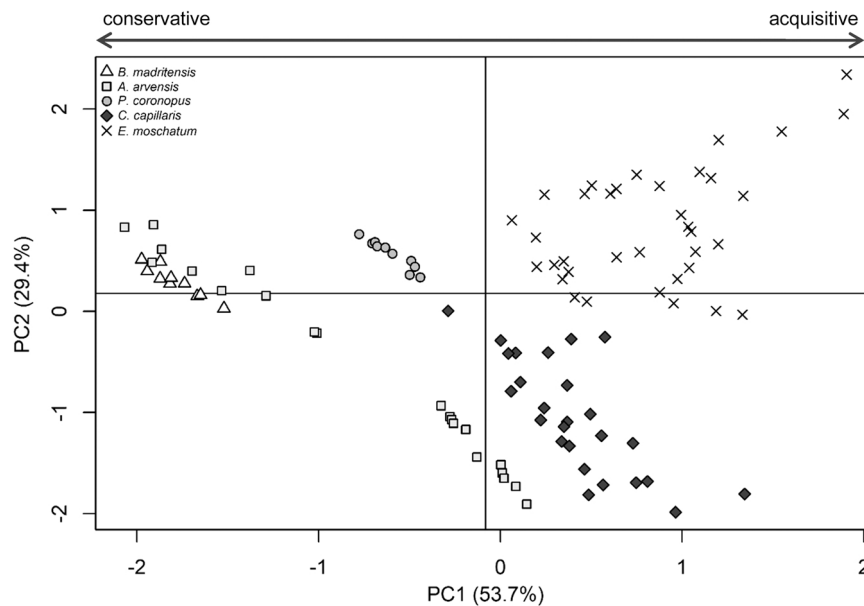
Fig. 1. Plant positions of the five study species at field control conditions (n = 10–36) on the plane defined by the two first PCA axes, according to four plant traits related with the Leaf Economics Spectrum (LDMC, leaf size, SLA and leaf N content). Variance explained by each component is specified in each axis title.

top chambers (OTC, Marion et al., 1997), which have been successfully used in global warming studies (e.g. Aragón-Gastélum et al., 2018; Buizer et al., 2012) inducing an elevation of 2–3ºC comparable to the temperature increment predicted for the Mediterranean region (IPCC, 2013). These chambers were 60 cm in diameter (ca. 94 cm^2 in area) and 40 cm in height and manufactured in methacrylate without UV filter to avoid any alteration of the characteristics of the light spectrum (Faberplast, Madrid). To mimic the reduction of precipitation, we placed rainfall-exclusion structures (2.5 ×2.5 ×1.5 m) composed of six methacrylate gutters, 0.14-m wide each, inclined 20º that captured 33% of rainfall, based on the design of Matías et al. (2012). To evaluate the effects of the combination of both climatic stressors ('W + D'), an OTC was located under the rainfall exclusion structure. Finally, control treatment units ('C') included plants in natural conditions. The experimental units that did not include OTC chambers (i.e. 'D' and 'C' treatments) had a similar area to that occupied by the OTCs (ca. 94 cm^2). The 18 plots including the experimental units of the four climatic treatments (W, D, W+D and C) were fenced to avoid the effects of grazing by livestock. The experimental units within a plot were installed as widely separated as possible (ca. 50 cm; Fig. S2).

In 2017 and 2018, we conducted two vegetation censuses: one of them coinciding with the flowering peak of species with early phenology (*A. arvensis* and *E. moschatum*) in late March-early April, and the other coinciding with the peak of species with late phenology (*B. madritensis*, *C. capillaris* and *P. coronopus*) in May. At the 72 experimental units (3 grasslands x 4 climatic treatments x 6 replicated plots), we measured the occurrence of the selected species using four 21 × 21 cm PVC quadrats (divided into nine 7 ×7 cm squares) that were placed randomly within each experimental unit and their position was marked in the soil for future censuses. Species frequencies were calculated from the number of squares where each species was present. In order to verify the differences created by climatic treatments, soil moisture at ca. 10 cm depth was measured monthly during both sampling years using a FP/MTS TDR probe (Easy-Test Technologies, Poland).

In order to describe the functional strategy of each species, leaves of each species were collected in the field and four above-ground traits were measured: leaf size, SLA (leaf area per unit of leaf dry mass), LDMC (dry mass per unit of water-saturated fresh mass) and leaf N content. Leaf size, SLA and LDMC were quantified following the procedures described by Garnier et al. (2001). Leaf size was determined using an

image analysis software (Image Pro-plus 4.5; Media Cybernetic Inc., Rockville, MD, USA) and N content was quantified in an external lab (INAN-EEZ-CSIC, Granada, Spain) using a CHN elemental analyzer (LecoTruSpec). These traits were measured in 7–10 plants per species and grassland under control conditions ('C') when plant material was enough to measure all the functional traits and analyzed together given their low expected intraspecific (Siefert et al., 2015) in relation to the interspecific variability. At the same time, 3–5 individuals of each species were randomly selected in those experimental units where they were present to measure a reproductive (number of fruits) and a vegetative (plant height) trait. Plant height was measured using a tape measure with precision of 0.1 cm. We also collected at least 20 fruits of each species by climatic treatment and grassland to obtain seeds for subsequent greenhouse experiments. Seeds were maintained in a chamber at 10 ºC until they were processed. A subset of 20% of the seeds was reserved to analyze differences in their mass, so that 50–200 seeds per treatment were weighted after being oven-dried at 60ºC for 48 h (Siddique and Wright, 2003). Seeds were weighted individually for *B. madritensis* and *E. moschatum*. For the other three species, seeds were gathered in groups of 10–20 due to their low weight under the readability (0.1 mg) of the analytical balance KERN ACS ACJ (KERN & Sohn GmbH, Balingen-Frommern, Germany).

2.3. Greenhouse experiments

The seeds collected from individuals subjected to the above-described climatic treatments in the field were germinated and grown under control conditions and the resulting plants were used to carry out two greenhouse experiments. One of them was aimed to test for transgenerational effects of the climatic treatments by exposing the offspring to the same treatments as their maternal plants. Thus, we can assess whether there is maintenance in the response to the climatic treatments or, by contrast, the two generations respond differently after being exposed to the same abiotic conditions. The second experiment was designed to test possible maternal environmental effects through exposure of the offspring to a common environment and analyzing if they showed contrasted responses depending on the climatic treatment of their maternal plants. Both experiments were conducted in the greenhouse facilities sited at the Center of Research, Technology and Innovation of the University of Seville (CITIUS, Seville, Spain).

2.3.1. Greenhouse experiment on transgenerational responses to climate change

Seeds of the five selected species from the field experiment in 2017 were pretreated by maintaining them in a cold room (10 °C) for one week to break dormancy and then placed in plastic trays at optimal conditions of temperature and moisture to stimulate germination. In mid-February 2018, emerged seedlings were transported to the greenhouse facilities and transplanted into plastic alveolar trays consisting of 60 holes (4 ×4×8 cm each) filled with a mixed substrate of 81% sand, 14% clay and 5% peat, simulating the edaphic texture of the study area. This substrate had been previously sterilized by autoclaving it at 121ºC for three consecutive days (McCarthy-Neumann and Kobe, 2010). In order to provide microbial communities naturally co-occur with the selected species, all the alveoli were irrigated with 20 ml of a soil microbial inoculum obtained after macerating 10 kg of soil collected at the study area in 20 l of distilled water over 2 days (Matías and Jump, 2014).

The plastic trays were placed in two greenhouse units following the same full factorial design as the field experiment (treatments 'C', 'W', 'D' and 'W+D'; see details above) so that 10–20 individuals per species from each climatic treatment in the field were exposed in the greenhouse to the same treatment of their maternal plant. These treatments were imposed by controlling air temperature and soil moisture. Air temperature was maintained at 24.5 ºC (oscillation day/night 15.9–27.7 °C) in one glasshouse unit and 27 °C (oscillation day/night 18.3–30.3 °C) in the other unit during the whole experiment, mimicking present conditions and future predictions of climate change models in the Mediterranean area. Mean air humidity was 65.2 ± 0.26 and $59.9 \pm 0.24\%$ for the two units, respectively. After transplantation, all the alveoli were irrigated daily with 15 ml of distilled water for one month to ensure seedling survival and adequate root development. After that, we controlled soil moisture by a differential irrigation whereby half of the alveolus were watered with 18 ml of water (according with the average rainfall conditions in spring at the study area; daily data obtained from meteorological station of Hinojosa del Duque for the 2008–2017 period), and the other half received 33% reduction (12.1 ml) over the previous treatment (simulating the drier conditions predicted for the next decades in the study area; Manabe et al., 2004). Eight months later, irrigation was stopped to simulate the dry period of Mediterranean summers. Soil moisture was measured monthly throughout the experiment in all the alveoli using a TDR probe (Easy-Test Technologies, Poland) (Fig. S3).

2.3.2. Greenhouse experiment on maternal environmental effects

Seeds of the species *C. capillaris*, *P. coronopus* and *B. madritensis* collected in the field in 2018 were pretreated and planted at the same conditions as in the climatic treatments experiment. These species were selected because, maintaining a wide range of functional strategies, they presented contrasted responses to climatic treatments (see results) and produced a sufficient number of seeds to be used in a new assay. Before beginning the experiment, seeds were classified according to the climatic treatment of their maternal plants. In April 2019, 25 seeds per species and maternal treatment were planted in alveolar plastic trays (see details in the previous section) in the greenhouse. All plants were subjected to optimal conditions (similar to climatic treatment 'C') for which an irrigation of 18 ml per alveoli and a temperature of 24.5 ºC (oscillation day/night 15.9–27.7 °C) was maintained throughout the experiment.

2.3.3. Plant monitoring and data collection

During the entire period of both greenhouse experiments, all experimental plants were monitored bi-weekly to have a complete record of their reproductive phenology. In each census, we counted the number of flowers and fruits of each individual and changed the location of the plastic trays to avoid possible effects of minor differences in environmental conditions. Once irrigation was cut off, the frequency of the census was changed to daily, noting the date of mortality of each plant for the calculation of the survival period (difference between the date of mortality and the date of emergence of each seedling; days). All plants were harvested when they completed their life cycle. Just before harvesting, we measured plant height with a ruler (precision 0.1 cm) and soil moisture with a TDR and we calculated lethal humidity (soil moisture at the moment of plant mortality; %). Once in the laboratory, above- (AGB) and below-ground (BGB) biomasses were separated, oven-dried at 60 °C for 48 h and then weighed with a precision balance. Finally, we calculated the root mass fraction (RMF, root dry mass per unit of total plant dry mass; g g^{-1}), which indicates the proportional biomass investment in the below-ground compartment.

2.4. Data analyses

For the functional characterization of the study species, the traits commonly associated to resource uptake (SLA, LDMC, leaf size and foliar N content) were related using Principal Component Analysis (PCA). The PCA was conducted analyzing the correlation matrix with 25 maximum iterations for convergence without rotation to extract independent PC factors with eigenvalues > 1. Data were standardized using z-score prior to PCA. The scores of the first component (PC1) were used for the description of the conservation (more negative values) - acquisition (more positive values) axis. This component explained a high percentage of variance (53.7%) and correlated all the variables, positively for leaf size, leaf N and SLA and negatively for LDMC (Table S1).

The effects of the experimental climatic treatments (W, D and W + D) on the eight response variables related with reproductive output (number of flowers and fruits), plant growth (plant height, AGB, BGB and RMF), plant survival (survival period and lethal humidity) and seed mass were assessed by General Linear Models (GLM) and Bonferroni-Dunn's test as post hoc analysis. We used air temperature and water availability as fixed factors and we included *grassland* as a random factor in the particular case of the field data. We conducted the same analysis on the response variables measured in the common environment experiment, but using maternal climatic treatments (air temperature and water availability on maternal plants) as fixed factors to identify possible maternal effects.

Phenotypic plasticity was calculated for each species as the variability of the response variables measured in the plants subjected to different climatic treatments in the greenhouse. Likewise, we calculated the phenotypic variability of the plants subjected to a common environment in relation to the maternal treatments from which the seeds were obtained. In both cases, we used the simplified relative distance plasticity index (RDPI$_s$) to calculate variabilities since it tolerates statistical comparisons of variability among species (Valladares et al., 2006). This index was calculated for each species and response variable as follows:

$$\text{RDPI}_s = \Sigma \left[d_{i \to j} / (x_i + x_j) \right] / n$$

Where $d_{i \to j}$ is the distance of a variable value between treatments i and j (treatments C, D, W and W+D), x are variable mean values and *n* are the number of distances. To evaluate interspecific differences of RDPI$_s$, we used One-Way ANOVA with species as grouping factor and Tukey test as post hoc analysis. The relationship between phenotypic plasticity and the functional strategy of the species was investigated using regression analysis (Pearson coefficient) between RDPI$_s$ and PC1 scores of the acquisition-conservation axis. To quantify the magnitude of the maternally inherited variability with respect to the variability due to climatic variability, we calculated a 'maternal index' from the difference between the variability (RDPI$_s$) of the plants coming from treated maternal plants but exposed later to a common environment and the variability of those plants directly subjected to climatic treatments in the greenhouse. Higher values of this index would indicate higher maternal effects relative to the intrinsic variability of the individuals.

Every statistical analysis was performed with IBM SPSS V. 26 for Windows using a significance level (α) of 0.05. Graphs were obtained with Sigma-Plot 14.5 for Windows, except for the PCA graph that was obtained with R-software (R Core Team, 2021). Prior to the use of parametric models, data series were tested for normality with the Kolmogorov-Smirnov's test, for homoscedasticity with the Levene's test and log-, inverse- or square-root-transformed when assumptions were not achieved.

3. Results

3.1. Responses to climatic treatments in the field

Climatic treatments applied in the field experimental plots induced temporal changes in plant species frequency. Thus, one of the most acquisitive species (*C. capillaris*) increased its frequency between 2017 and 2018 but it did it to a lesser extent in the experimental units subjected to experimental climate manipulation (i.e. W, D and W+D treatments) than under control conditions (C). On the contrary, the species with foliar traits more related to a conservative resource-use strategy (*B. madritensis*) was the only one that significantly reduced its frequency between both years (Fig. 2). When we analyzed each year separately, we did not find differences among climatic treatments in species frequencies in 2017 or 2018 (GLM, P > 0.05).

In terms of plant responses to climatic treatments, significant changes were detected for the vegetative (plant height) but not for the reproductive (number of fruits) traits. The effects of warming and drought presented opposite trends. Thus, experimental warming induced height increases in three of the studied species, with the most acquisitive species (*E. moschatum*) being the only one that kept this response during the two sampling years. In contrast, experimental drought reduced the size of the two Asteraceae species (*A. arvensis* and *C. capillaris*; Table 1), but this effect only took place after two years of climate manipulation (i.e. 2018 year; Table 1). The weight of the seeds collected for the subsequent experiments did not show significant differences in the climatic treatments imposed for any of the studied species in any of the two harvest years (GLM, P > 0.05).

3.2. Transgenerational responses to climatic treatments (greenhouse experiment 1)

Important interspecific differences were found in the responses of the five study species to climatic treatments under greenhouse conditions.

These responses to one or both climatic stressors were essentially a decrease in aerial biomass, a reduction in the survival period and an increase in the soil water content at which plant mortality occurs (Fig. 3A, C and D). Nevertheless, the most conservative species (*B. madritensis*) was the only one that did not show any significant negative effect associated with these climatic factors. On the contrary, it experienced increases in its vegetative growth (plant height) and reproductive output (production of flowers and fruits) in response to drought. In contrast, species with a more resource-acquisition strategy (i.e. *C. capillaris*) were more sensitive to warming and, especially to drought, reducing their biomass production and survival and increasing their lethal humidity at higher levels of abiotic stress (Table 2). In terms of reproductive output, the production of flowers and fruits was stimulated in two of the species with late phenology (*P. coronopus* and *B. madritensis*) in response to one or both climatic factors, whereas *C. capillaris* decreased it reproductive output under more stressful conditions (Table 2, Fig. 3B).

The magnitude of the responses was also contrasted among species, so that they exhibited different phenotypic plasticities. Consequently, the most conservative species showed the lowest mean phenotypic plasticity value when combining the 9 response variables (RDPI$_s$ = 15.5 ± 4.7%), whereas one of the most acquisitive species (*C. capillaris*) presented the highest RDPI$_s$ mean value (45.2 ± 8.8%; t = −2.194, P < 0.05). However, no significant relationship was found between the functional strategy (PC1 scores, Table S1) and phenotypic plasticity (RDPI$_s$; Pearson regression coefficient, P > 0.05) given the high variability of the different response variables and the opposite trend that exhibited the most acquisitive species (*E. moschatum*), which showed a lower value of mean plasticity (27.5 ± 6.2%).

3.3. Maternal environmental effects (greenhouse experiment 2)

Some of the effects that climatic treatments induced on the species were maternally transmitted to their offspring, since they showed differences in the measured variables in relation to their maternal treatments despite being subjected to homogeneous optimal conditions (Table 3). These maternal effects were very uneven for the three selected species. Lower values of biomass, height and survival and higher lethal humidity were registered for the offspring of maternal plants of the most conservative species (*B. madritensis*) subjected to both warming, drought and the combination of both abiotic stressors. On the contrary, maternal effects were much less marked in the most acquisitive species (*C. capillaris*), which only showed a positive response (greater survival
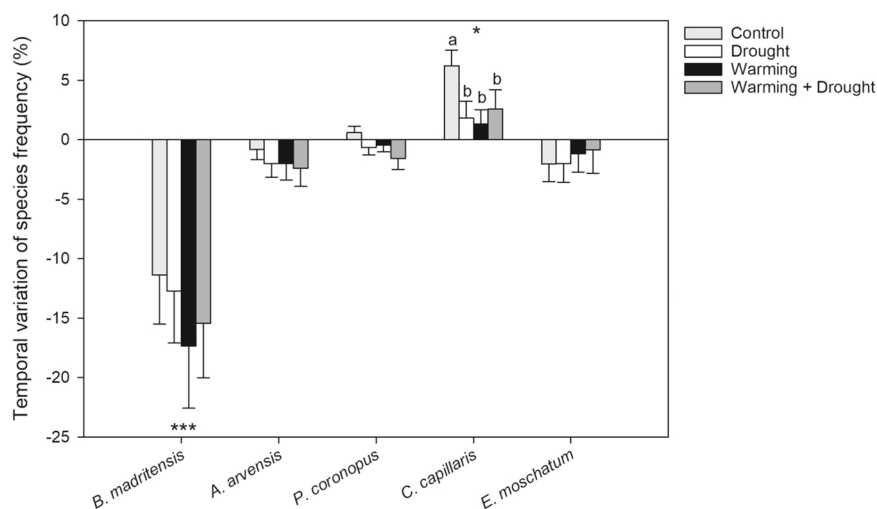

Fig. 2. Difference of frequency between spring 2017 and 2018 for the five study species at the three climatic treatments (D, W and W+D) and the control (C) units set in the field. Significant changes are designated with asterisks (*P < 0.05; **P < 0.01; ***P < 0.001). Different letters indicate differences among treatments for a given species. Species have been arranged from more conservative to more acquisitive strategy.

Table 1

Results of the GLM that tested the effect of warming (W), drought (D) and their interaction (W*D) on the height of the plants located at the experimental units in the field. The proportion of the explained variance (SSx/SStotal) and the level of significance (*P < 0.05; **P < 0.01; ***P < 0.001) of each factor are indicated for the five study species. Significant effects of the climatic factors have been highlighted in bold letters and the sign of the effect is specified in brackets. Species have been arranged from more conservative to more acquisitive strategy.

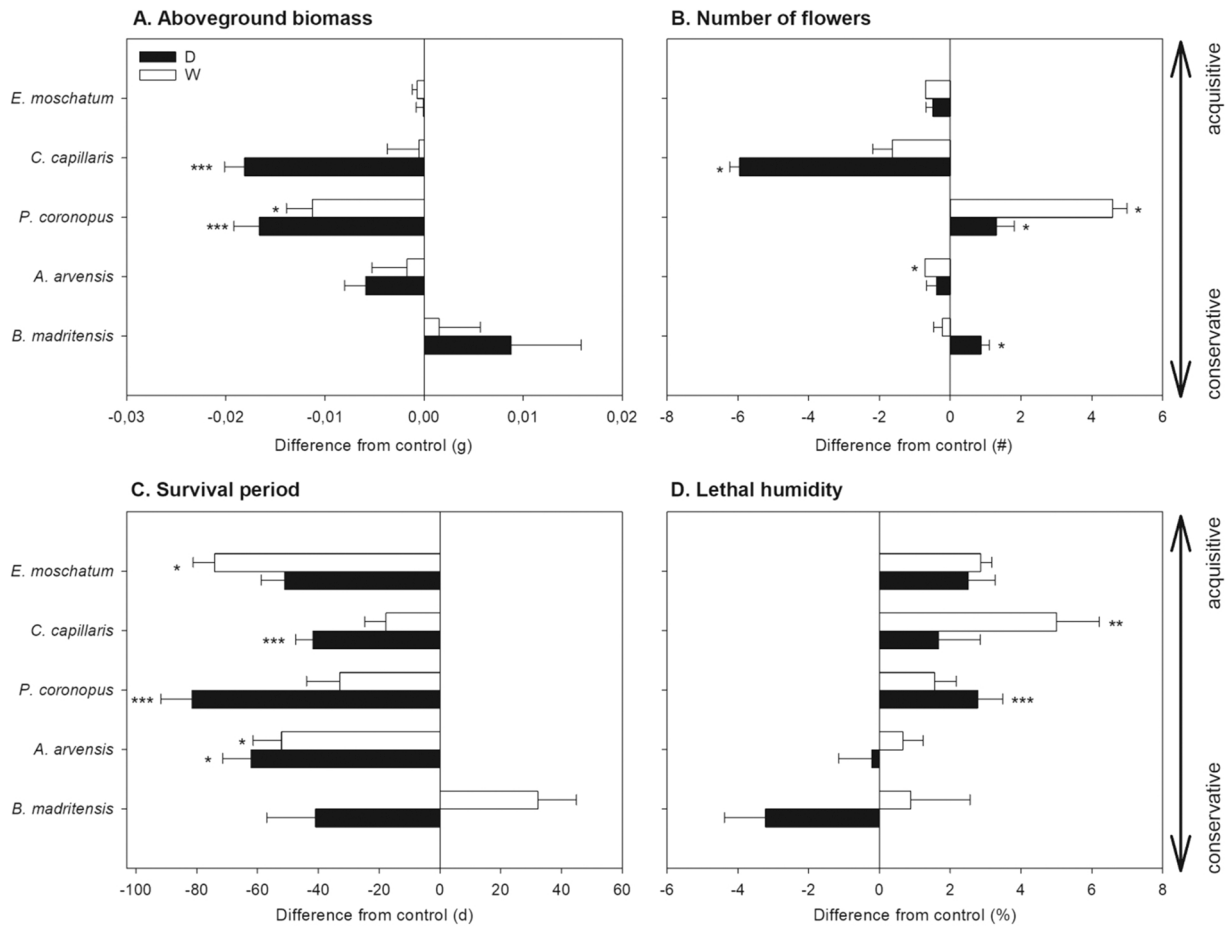|		*B. madritensis*		*A. arvensis*		*P. coronopus*		*C. capillaris*		*E. moschatum*	
	Year	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Height (cm)	N	155	140	47	60	20	17	150	169	137	159
	W (°C)	**2.75*(+)**	0.03	0.03	**6.53*(+)**	–	9.34	1.55	0.29	**4.90*(+)**	**4.27* *(+)**
	D (%)	2.32	2.41	5.71	**16.37* *(-)**	1.25	0.02	1.21	**2.50*(-)**	1.76	0.42
	W*D	**3.26***	0.39	**9.07***	**12.21***	–	–	0.49	1.64	0.65	0.52

Fig. 3. Changes in aboveground biomass (A), number of flowers (B), survival period (C) and lethal humidity (D) in response to increased temperature (W; warming) and reduced water availability (D; drought) in relation to control conditions. Significant changes are labelled with asterisks (*P < 0.05; **P < 0.01; ***P < 0.001) according to Bonferroni-Dunn's tests. Horizontal bars represent standard-error values.

period) in plants coming from maternal individuals subjected to drought (Table 3).

These inter-specific differences in the magnitude of the maternal effects induced by warming and/or drought conditions was reflected by the maternal index, appearing predominantly positive values for most of the traits measured in *B. madritensis* and negative values for those quantified in the other two species (specially for *C. capillaris;* Fig. 4).

4. Discussion

The results of our study suggest that transgenerational responses to cues directly related to climate change (warming and drought) differently influence vegetative and reproductive output of annual plant species with contrasting functional strategies. Thus, the direct effects of the abiotic stressors on maternal plants were retained or new ones

appeared in their offspring, with special impact on one of the most acquisitive species (*C. capillaris*). On the contrary, the most conservative species (*B. madritensis*) highly tolerated increased temperature and limited water availability but important deleterious maternal effects were identified, given the differences in traits measured in descendants of maternal plants subjected to different abiotic conditions.

4.1. Are warming and drought-induced effects on performance of acquisitive species quantitatively higher than those on conservative ones?

Climatic treatments applied in our field trial affected plant community composition. Thus, one of the most resource-acquisitive species (*C. capillaris*) significantly increased its relative frequency when it was not under the influence of experimental warming and / or drought. This result is in accordance to the general statement that species exhibiting a

Table 2

Results of the GLM that evaluated the effect of warming (W), drought (D) and their interaction (W*D) on the 9 response variables of the plants at the greenhouse experiment. The proportion of the explained variance (SSx/SStotal) and the level of significance (*P < 0.05; **P < 0.01; ***P < 0.001) of each factor are indicated for the five study species. Significant effects of the climatic factors have been highlighted in bold letters and the sign of the effect is specified in brackets. Species have been arranged from more conservative to more acquisitive strategist.

Trait	B. madritensis			A. arvensis			P. coronopus			C. capillaris			E. moschatum		
	W (°C)	D (%)	W*D	W (°C)	D (%)	W*D	W (°C)	D (%)	W*D	W (°C)	D (%)	W*D	W (°C)	D (%)	W*D
AGB (g)	0.12	1.78	5.59	0.19	11.08	1.55	9.47* (-)	20.23*** (-)	10.60* (-)	0.00	30.95*** (-)	3.70	0.75	0.06	0.12
BGB (g)	3.39	0.01	4.20	1.38	13.69	2.98	25.57*** (-)	9.59* (-)	12.97* (-)	2.18	2.24	2.20	0.12	6.87	0.28
RMF	1.37	0.92	1.01	11.66	11.09	10.49	6.31	1.75	1.08	2.53	2.33	2.42	0.77	0.40	0.02
Height (cm)	4.54	14.31* (+)	1.07	14.15	3.50	4.39	4.47	0.35	3.83	0.38	17.34* (-)	0.53	6.46	0.33	8.18
Root length (cm)	4.17	1.60	0.23	0.01	1.26	1.22	0.68	2.64	3.53	10.64	4.41	0.33	5.70	19.06* (-)	28.53* (+)
Flowers (#)	1.98	20.78* (+)	1.77	8.80	0.98	0.98	69.74*** (+)	6.46* (+)	2.67* (+)	2.52	44.58***	6.47* (-)	3.07	0.81	0.81
Fruits (#)	0.27	17.54* (+)	0.21	8.80	0.98	0.98	68.25** (+)	7.58*** (+)	2.16	2.43	43.27***	6.30* (-)	0.00	0.00	–
Survival period (d)	4.46	6.83	2.07	12.30* (-)	16.85* (-)	0.79	5.52	28.20*** (-)	33.39*** (-)	4.89	27.18*** (-)	2.29	11.85* (-)	1.19	2.77
Lethal humidity (%)	0.75	3.90	8.17	5.14	0.05	0.87	0.75	19.15*** (+)	33.11*** (+)	25.30** (+)		2.88	2.82	1.49	0.13

resource-acquisition strategy tend to predominate in communities of ecosystems with favorable conditions, being however more susceptible than conservative ones to biotic or abiotic stress (Kandlikar et al., 2020; Pérez-Ramos et al., 2017; Reich, 2014). Mild conditions promote acquisitive phenotypes because they exhibit superior competitive ability due to their rapid growth rates and favorable traits such as larger leaves and plant height, while conservative species better tolerate harsh conditions but their competitiveness is limited (Midolo and Wellstein, 2020; Read et al., 2014).

In order to record an integrative measure of plant fitness (Díaz et al., 2016), we measured the maximum height of experimental plants in the field. Plant growth increments with temperature have been frequently observed as a consequence of the improvement of biochemical processes such as photosynthesis or respiration (Way and Oren, 2010) and it was a generalized response recorded in our field trial for the species with both acquisitive and conservative leaf traits. However, the species with the most acquisitive strategy was the most sensitive to temperature, showing significant height increases in the two sampled years. This temperature-induced effect on plant height has been described as especially important in fast-growing species (Poorter and Garnier, 2007; Wright and Westoby, 2001). Nonetheless, Grady et al. (2013) also recorded a relation between growth rate and conservative leaf traits at high temperatures, being explained by the greater importance of hydraulic architecture than leaf-level photosynthesis to regulate growth in these species under warmer conditions. On the contrary, drought conditions induced the opposite effect in our experimental plants, reducing plant height in one of the most acquisitive species (C. capillaris). This is in accordance with the expected greater sensitivity of acquisitive species to environmental stress and it is a broadly-known response to reduced water availability in Mediterranean grasslands species (Pérez-Ramos et al., 2019, 2012). Our results therefore indicate a tendency for acquisitive herbaceous species to be more affected by climatic effects associated with global warming, resulting thus in a predominance of conservative species in Mediterranean herbaceous communities with the forecasted intensification of this phenomenon. However, studies with a larger number of species and a wider range of functional strategies are necessary to confirm the observed trend and elucidate whether the patterns detected for some annual Mediterranean species can be extended to other biomes and plant life-forms.

4.2. Do species with different resource-use strategies show different transgenerational responses to contrasting climatic conditions?

In addition to plant height, the rest of the variables not covered in the previous section (plant biomass, reproductive output and survival) were measured in the greenhouse experiment on transgenerational responses to climate change. This experiment was applied on the descendants of the field trial plants, so that transgenerational influence on their responses could be assessed. Results from this greenhouse experiment confirmed the aforementioned trend detected under field conditions by which the most conservative species (*B. madritensis*) was only positively affected by climatic factors, while one of the most acquisitive species (*C. capillaris*) was the most negatively affected (especially by drought). This tendency was evidenced by the greater passive (detrimental) phenotypic plasticity exhibited by *C. capillaris* in comparison with *B. madritensis*. In this sense, *B. madritensis* continued to show a stimulation and *C. capillaris* a reduction of height growth after two generations exposed to climatic conditions, being this a persistent transgenerational effect. On the contrary, *A. arvensis*, whose growth was limited by drought in parental plants, did not show this effect when the descendants were exposed to the same climatic conditions. Adaptive transgenerational effects could have taken place in this case, thus mitigating the negative responses to abiotic stressors of the parental plants in their offspring (Colicchio and Herman, 2020; Galloway and Etterson, 2007; Herman and Sultan, 2011; Mousseau and Fox, 1998). Accordingly to our results, Herman et al. (2012) found that the annual

Table 3

Results of the GLM that tested maternal effects on the 7 variables measured on plants grown in a common environment from seeds of maternal plants under climatic treatments (W, warming; D, drought and W*D, their combination). The proportion of the explained variance (SSx/SStotal) and the level of significance (*P < 0.05; **P < 0.01; ***P < 0.001) of each factor are indicated for the three selected species. Significant effects of the climatic factors have been highlighted in bold letters and the sign of the effect is specified in brackets. Species have been arranged from more conservative to more acquisitive strategist.

| Trait	B. madritensis			P. coronopus			C. capillaris		
	Maternal W (°C)	Maternal D (%)	Maternal W*D	Maternal W (°C)	Maternal D (%)	Maternal W*D	Maternal W (°C)	Maternal D (%)	Maternal W*D
AGB (g)	**9.99* ** (-)**	**19.87* ** (-)**	**18.35* ** (-)**	0.03	0.66	1.85	3.89	4.06	5.52
BGB (g)	**8.02* ** (-)**	**29.90* ** (-)**	**18.49* ** (-)**	1.64	**9.86* (-)**	0.17	6.30	0.54	6.64
RMF	0.91	1.17	0.31	3.33	0.03	6.13	2.10	7.81	0.00
Height (cm)	**11.76* ** (-)**	**36.19* ** (-)**	**10.50* ** (-)**	**26.86* ** (-)**	0.25	6.68	3.46	4.90	8.10
Flowers (#)	0.50	0.01	3.32	3.04	3.04	5.31	2.29	2.29	3.23
Survival period (d)	**10.89* ** (-)**	**14.18* ** (-)**	**4.99* (-)**	0.12	0.03	**11.20* (+)**	3.35	**13.03* (+)**	2.93
Lethal humidity (%)	**10.49* ** (+)**	**13.04* ** (+)**	**10.94* ** (+)**	0.57	0.51	0.34	4.16	7.19	5.00
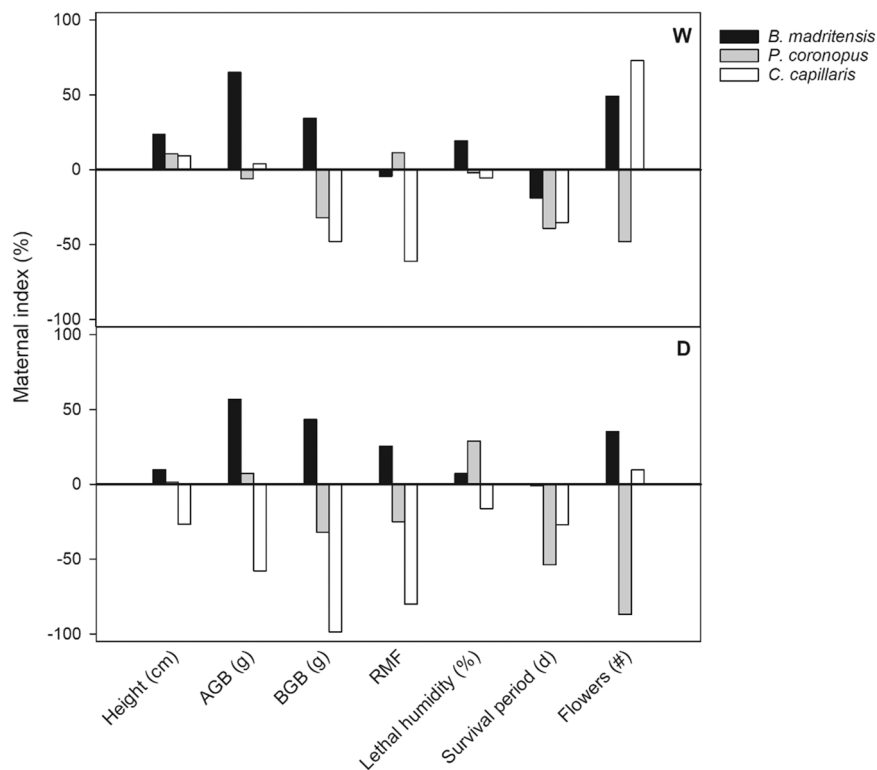
Fig. 4. Difference between the variability of the plants in relation to the treatments of their maternal plants and the variability of the plants directly treated ('maternal index') for the seven response traits measured in the three study species for the warming (W) and drought (D) factors.

Polygonum persicaria showed adaptive transgenerational responses improving its growth after being exposed to drought for 2 generations. They also found an increment of survival after 3 generations under the same conditions, an effect that we did not observe in our 2-generations study. However, this rapid improvement of fitness across generations could point to a better long-term performance with subsequent enhanced survival period.

In relation to reproductive output, species with a conservative tendency exhibited stimulations of flower and fruit production associated to both climatic cues, a fact that confirms their potential higher ability to cope with stressful conditions compared with acquisitive species. On the one hand, increasing temperature affects plant phenology frequently advancing the first flowering (Bock et al., 2014) and may lengthen the duration of the reproductive period (Pérez-Ramos et al., 2020; Ziello et al., 2012), thus increasing the final quantity of flowers and fruits produced. On the other hand, reduced water availability usually limits

the number of flowers by hindering floral expansion and turgor due to high evapotranspirational demand (Galen et al., 1999) and leading to fewer resources available for reproduction (Phillips et al., 2018). This was the case of the acquisitive species *C. capillaris*, which reduced its reproductive output under drier conditions after two generations. Instead, the two most conservative species (*P. coronopus* and *B. madritensis*) exhibited higher reproduction under these drought-stressed conditions. This may be related to a Drought Escape type of adaptation by which some plants advance their flowering in the face of terminal droughts (Kooyers, 2015), promoting higher reproductive yields. This type of adaptation has been described in annual plants, especially in Mediterranean-type ecosystems (Shavrukov et al., 2017), which make it suitable for our studied species.

The greater tolerance of the most conservative species to climatic stressors, as explained in detail above, likely allowed them to maintain its survival period across climatic treatments for a longer time, while the

rest of the species accelerated their mortality under warmer or drier conditions. Thus, *A. arvensis* and *E. moschatum* maintained their growth rates and reproductive output irrespectively of the climatic treatment, but survived a shorter time period under the simulated scenarios of climate change. This indicates that there were other processes not measured in this study that were shortening individual survival, probably promoted by physiological or biochemical mechanisms (León-Sánchez et al., 2020). Pérez-Ramos et al. (2013) also found that drought-survival of species with conservative foliar traits was favored by their greater capacity to delay foliar dehydration than acquisitive species, exhibiting higher leaf water potential and relative water content under more stressful conditions.

4.3. Does phenotypic variability in the response of the maternal plants influence the phenotype of their offspring depending on the resource-use strategy of the species?

Maternal effects can negatively affect offspring performance or improve it by adapting their phenotypes to maternal environmental conditions, thus being a particular type of adaptive transgenerational plasticity (Marshall, 2008). However, to our knowledge, this has not been tested in plants with different functional resource-use strategies. In our study, maternal effects showed opposite trends in species with contrasted functional strategies, being low and adaptive for the acquisitive *C. capillaris* while high and deleterious for the conservative *B. madritensis*. Thus, under optimal conditions, *C. capillaris* plants showed higher survival period when their maternal plants had been previously exposed to drought. On the contrary, *B. madritensis* individuals accumulated less biomass, grew less and died before under higher soil moisture when their maternal plants had been exposed to warming or drought, finally showing higher mean maternal index than *C. capillaris*. Transgenerational effects in opposite directions were also registered by Sultan et al. (2009), in this case in congeners of *Polygonum*, with the most generalist species experiencing adaptive responses to drought while the species more adapted to moist soils reduced its performance in the descendants of stressed plants. This is evolutionary important since it contributes to the ecological distancing among species, by intensifying the difference in environmental tolerance among them in subsequent generations.

Previous studies have shown that maternal effects are usually transitory and disappear along the first generation of plants under homogeneous and different conditions to the mother plant (Agrawal, 2002; Gianoli, 2002). When these effects persist even in homogenous conditions as in our case, some type of non-genetic differentiation mediated by the maternal environment has probably taken place in the offspring (Bischoff and Müller-Schärer, 2010). In particular, changes in seed provisioning (carbohydrate, lipid, protein, and mineral nutrient reserves of the seed) have been identified as a main driver of maternal effects on fitness-related traits (Wulff, 2018). However, as we did not find effects of the climatic treatments on the weight of the seeds produced, it is not expected that this mechanism was the main responsible of the observed effects (Dyer et al., 2010). Likewise, other seed-mass independent mechanisms controlling maternally-transmitted responses have been identified, such as changes in hormones and protein contents in seeds and epigenetics (reviewed in Herman and Sultan, 2011). For example, changes in abscisic acid (ABA) concentration in seeds from maternal plants subjected to drought conditions (Arnold et al., 1991; Kalandyk et al., 2017) might have repercussions on the fitness of the emergent plant under stressful conditions throughout its entire life-cycle (Cutler et al., 2010; Peleg and Blumwald, 2011). Higher ABA contents have been related to the ability to develop conservative strategies in the face of drought (Wilkinson et al., 2012), so its transgenerational alteration could modify this drought-tolerant strategy. More recently, epigenetic changes, such as DNA methylation, changes in small RNA or histone modifications, have been pointed as important inductors of maternal effects that can be inherited though multiple generations (Herman and Sultan, 2011; Holeski et al., 2012). Transgenerational epigenetics have been specially studied in the *Arabidopsis* model and some crop species (Hauser et al., 2011; Quadrana and Colot, 2016) but, to our knowledge, no studies linking its prevalence with different functional types of plants have been conducted. Further analysis including hormones quantification (Vu et al., 2015) or cytosine-methylation (Chano et al., 2021) would allow to better understand the underlying mechanisms that explain these opposite persistent maternal effects in species with contrasting functional strategies.

5. Conclusions

Our findings indicate that, in the face of the warmer and drier conditions expected for the Mediterranean area, plant species with different resource-use strategies would respond differently both within and across generations, affecting their growth and reproductive output and, consequently, altering plant community composition. According to our results, the higher sensitivity of fast-growing herbaceous species could be considered a disadvantage compared to slow-growth, stress-tolerant species in a scenario of intensified climate change. The predominance of conservative species in the face of stressful climatic conditions has been previously observed in Mediterranean shrubland communities, in a study that analyzed changes in species composition over 9 consecutive years (Pérez-Ramos et al., 2017). However, in the case of herbaceous species, this effect could be dampened by an uneven transgenerational transfer, as we found that species that highly invest in tolerating these more stressful environments may be undergoing other processes that negatively affect its performance in subsequent generations when conditions would become milder. These transgenerational effects have been described as more relevant in annual species (Yin et al., 2019), explaining the greater contribution of this process in the studied species than in the shrubs of the same climatic region.

Our study shows the importance of analyzing responses to stress across generations to understand the long-term performance of different functional species, since their expected responses can be modified depending on parental influence. Likewise, the observed trend opens up a valuable line of research to provide information on the responses of herbaceous plant communities under future climate change scenarios. Further studies including a higher number of species with opposite functional strategies and biochemical and epigenetic studies would be complementary to accurately infer their contrasting transgenerational transference in response to climatic stress.

CRediT authorship contribution statement

IMP-R and JC designed the study; MDH-G, AV-G, MM-M and IMP-R collected the data; BG-T analyzed the data and wrote a first draft, with revisions by IMP-R, JC and MDH-G. All authors approved the final version of the text. BG-T: Blanca Gallego Tévar; MDH-G: María Dolores Hidalgo-Gálvez; JC: Jesús Cambrollé; MM-M: Marcelino Martínez-Muñoz; AV-G: Alejandro Villar-Godoy; IMP-R: Ignacio M. Pérez-Ramos.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2022.104817.

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