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RESEARCH ARTICLE

Range-wide intraspecific variation reflects past adaptation to climate in a gypsophile Mediterranean shrub

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Abstract

1. Phenotypic differences among populations stem from the interaction between neutral and adaptive processes, and phenotypic plasticity. Although clinal trait variation along climatic gradients often evolves in widely distributed species, it is unknown whether substrate specialization, such as that of Mediterranean gypsum plants, has constrained adaptation to climate.
2. Using a common garden experiment with two contrasting watering treatments, we quantified phenotypic plasticity, assessed evidence for footprints of selection using $F_{ST} - Q_{ST}$ comparisons, and evaluated the ecological factors driving genetically based phenotypic differentiation of 11 populations encompassing the full environmental range of the gypsum shrub *Lepidium subulatum*.
3. We found evidence for genetic differentiation among populations related to climatic differences, with populations from warmer and drier sites showing lower specific leaf area and leaf N, earlier phenology, greater water use efficiency and greater fitness. Multiple lines of evidence suggest that this differentiation was driven by past divergent selection rather than neutral processes. All populations showed high phenotypic plasticity, indicating that plasticity has not been selected against, even in populations from sites with harsher climatic conditions.
4. *Synthesis*. Our results indicate that despite strong substrate specialization, adaptive differentiation related to climatic gradients occurs in this species. However, we also found that populations from mesic sites may be particularly vulnerable to future climate change given their relatively lower fitness under both wet and dry conditions.

KEYWORDS
adaptive intraspecific variation, divergent evolution, gypsophiles, local adaptation, natural selection, phenotypic plasticity, population differentiation, $Q_{ST} - F_{ST}$ comparisons

1 | INTRODUCTION

Intraspecific phenotypic variation is widely found in nature, and understanding its causes and consequences is key to predicting the evolutionary trajectories of populations (Bolnick et al., 2011; Merilä & Crnokrak, 2001; Westerband et al., 2021). Phenotypic differences among populations result from the complex interaction between genetic differentiation, driven by adaptive evolution and/or neutral evolutionary processes such as migration and genetic drift, and differences in environmentally induced phenotypic responses, that is, phenotypic plasticity (Merilä & Crnokrak, 2001; Pigliucci, 2001; Westerband et al., 2021). Widely distributed species often occur across broad environmental gradients, which likely impose differential selective pressures across populations (Blanquart et al., 2013; Ramírez-Valiente et al., 2010). If there is sufficient genetic variation and the effects of natural selection are stronger than those from neutral evolutionary processes, differences in patterns of selection may lead to adaptive population differentiation (Blanquart et al., 2013; Merilä & Crnokrak, 2001).

Phenotypic plasticity may also contribute to differentiation among populations (Mitchell-Olds et al., 2007; Pigliucci, 2001; Westerband et al., 2021). Specifically, plasticity may allow the expression of optimum phenotypes in response to contrasting environmental conditions, preventing the adaptive evolution of traits (see e.g. Oplaat & Verhoeven, 2015; Sultan & Matesanz, 2015). In addition, although epigenetic mechanisms are known to play a prominent role in the plastic responses of organisms, plasticity also has a genetic basis and can evolve by natural selection. Consequently, populations inhabiting contrasting environmental conditions may differ in their plastic responses (Pigliucci, 2001; Valladares et al., 2007). Determining whether phenotypic differentiation results from past adaptation, differences in plasticity, or a combination of both processes, is crucial to understand the potential adaptive differences of populations to new environmental conditions and make robust predictions on their long-term persistence (Franks et al., 2014; Welles & Funk, 2021).

In Mediterranean plant populations, precipitation, and temperature are the most critical factors for plant establishment, particularly during summer and in the first years after seedling emergence (Blondel et al., 2010; Matesanz & Valladares, 2014; Ramírez-Valiente et al., 2022). To cope with abiotic stress, Mediterranean plants have evolved functional syndromes associated with different drought-related strategies (Kooyers, 2015; Volaire, 2018). At the intraspecific level, widespread Mediterranean species experience substantial variation in temperature and drought intensity and duration across their distribution ranges (Matesanz, Ramos-Muñoz, Blanco-Sánchez, et al., 2020b; Ramírez-Valiente et al., 2022) that may have also driven the divergent evolution of populations. Specifically, it has been suggested that a less plastic, conservative syndrome associated with a drought-tolerant strategy would be favoured under harsher climatic conditions to cope with drought, whereas an acquisitive, drought-escape strategy with high plasticity would be favoured under more mesic environments to exploit favourable periods of higher water availability (e.g. Csilléry et al., 2020; Solé-Medina et al., 2022; Valladares et al., 2000; Van Kleunen & Fischer, 2005). However, most of this evidence comes from tree studies, with comparatively fewer studies focusing on patterns of population differentiation in Mediterranean perennial shrubs (e.g. Lázaro-Nogal et al., 2016; Matesanz, Ramos-Muñoz, Blanco-Sánchez, et al., 2020b). Therefore, whether Mediterranean perennial shrubs have responded to climatic heterogeneity via evolution through natural selection and/or phenotypic plasticity is not well understood.

Substrate specialization may also be a strong evolutionary force in plant populations. Together with drought and high temperatures, Mediterranean gypsophiles—plants restricted to gypsum soils—are subjected to chemical and nutrient imbalances (Cera et al., 2021; Escudero et al., 2015; Palacio et al., 2022) that impose highly restrictive conditions for plant growth and development. Specifically, gypsum (calcium sulfate dihydrate; $CaSO_4 \cdot 2H_2O$) soils have high calcium and sulfate but low nutrient availability, making S concentration a potential nutritional and physiological requirement for gypsophiles in natural conditions (Cera et al., 2021; Palacio et al., 2022). Therefore, differences in soil chemical composition across populations may also lead to divergent evolution in these habitat specialists. Alternatively, it has been proposed that substrate specialization may result in a common phenotype that is able to thrive in a restrictive environment (i.e. a stress resistance syndrome; Rajakaruna, 2018; von Wettberg et al., 2014). Accordingly, edaphic specialists may show low within- and among-population genetic variation, and may thus represent evolutionary dead-ends with limited evolutionary potential (Anacker et al., 2011; Rajakaruna, 2018; but see Blanco-Sánchez et al., 2023). To the best of our knowledge, the ecological factors that have promoted adaptive evolution in gypsophiles, and more importantly, whether adaptation to extreme edaphic conditions has limited the ability of gypsophile populations to respond to other selective pressures such as climatic variation, are currently unknown.

In a climate change scenario, assessing whether genetic differentiation is associated with past adaptation to climatic differences among populations will improve our ability to identify potentially vulnerable and resilient populations to future climatic conditions (Anderson & Song, 2020; Franks et al., 2014; Solé-Medina et al., 2022). A particularly useful approach to assess the role of past selection and neutral processes on the genetically based phenotypic differentiation of populations is $Q_{ST} - F_{ST}$ comparisons. Q_{ST} quantifies population genetic differentiation in phenotypic traits caused by both neutral and adaptive evolutionary processes, while F_{ST} estimates population differentiation in molecular markers driven only by neutral processes. Significant differences between these metrics evince footprints of selection (Leinonen et al., 2013; Merilä & Crnokrak, 2001; Whitlock, 2008). Furthermore, assessing the potential association between populations' phenotypes—expressed under common conditions— and their local environmental conditions is crucial to identify the selective pressures driving adaptive phenotypic differentiation (Blanquart et al., 2013; Brouillette et al., 2014; Ramírez-Valiente et al., 2022). Understanding whether environmental gradients drive genetically based phenotypic differences among populations has remained a key topic for evolutionary ecologists for decades (Gregor, 1944; Huxley, 1938; Turesson, 1922). A gradual phenotypic change across populations associated with an environmental gradient (i.e. an environmental cline) suggests that past adaptive evolution has occurred in response to the environmental variation (Brouillette et al., 2014; Ramírez-Valiente et al., 2022). In addition, since the expression of quantitative genetic variation may be environmentally dependent, i.e. adaptive population differentiation may be expressed in one environment while being absent in another, assessing how population differentiation varies across ecologically meaningful environments that reflect natural variation in environmental conditions is key to understanding adaptive differentiation (Cooper et al., 2022; Pigliucci, 2001; Ramírez-Valiente et al., 2018). Finally, since natural selection rarely acts on isolated traits but, rather, on a multivariate phenotype, evaluating the patterns of multivariate phenotypic variation and their association with environmental factors is needed to gain a deep understanding on the adaptive processes in natural populations (Solé-Medina et al., 2022 and references therein).

Our study assessed the role of past natural selection on quantitative genetic differentiation across populations and patterns of phenotypic plasticity to drought in the Mediterranean gypsum shrub *Lepidium subulatum*. We evaluated whether selection events, acting on populations' mean phenotypes and/or their plasticity, were likely driven by differences in climate and/or soil composition among populations. We sampled 11 populations throughout the entire environmental distribution range of the species, genotyped them using species-specific nuclear microsatellites, and characterized genetically based phenotypic differentiation across populations. We used an outdoor common garden experiment with two ecologically meaningful watering treatments that reflected natural spatiotemporal variation in water availability across the study populations. Specifically, we addressed two main questions: (i) Do populations of *L. subulatum* show genetically based phenotypic differences reflecting past adaptation to local climate and/or soil composition? (ii) Does *L. subulatum* express phenotypic plastic responses to drought in key functional traits, and, if so, do plastic responses vary among populations? We hypothesize that climatic differences across populations have driven adaptive population differentiation in *L. subulatum*. Alternatively, soil chemical composition may have played a more important role than climate in the evolution of gypsophiles, favouring a common stress resistance syndrome across populations. Finally, we expect lower phenotypic plasticity in populations with climatically harsher (i.e., warmer and drier) environmental conditions.

2 | MATERIALS AND METHODS

2.1 | Species description and sampled populations

Lepidium subulatum L. (Brassicaceae) is a small perennial shrub (20–60 cm high) endemic to the Iberian Peninsula and North Africa. It is one of the most dominant and widespread gypsophiles in the Iberian gypsum habitats, where it forms large populations. The species also shows rare occurrences in North Africa, with a few scattered and small populations known in Morocco and Argelia (M. Chambouleyron, personal communication). Individuals produce numerous small fruits (silicles), each containing two seeds (Hernández Bermejo & Clemente, 1993). *Lepidium subulatum* has low seed dispersal ability, and is predominantly outcrossing with partial self-compatibility, as supported by low inbreeding coefficients throughout its range (Blanco-Sánchez et al., 2021). Although gene flow across populations is higher in outcrossing compared with selfing species (Aguilar et al., 2008; Honnay & Jacquemyn, 2007), adaptive population differentiation may occur in the presence of gene flow when selection is strong and constant (Jump & Peñuelas, 2005).

In June 2017, we sampled 11 populations across the Iberian Peninsula. The selected populations covered the worldwide climatic distribution of *L. subulatum*, spanning a wide gradient of climatic conditions among populations (Table 1; Figure 1; Table S1), with substantial differences in annual precipitation (328–580 mm) and annual mean temperature (11.6–17.1°C). Climatic data were extracted from WorldClim bioclimatic layers (Fick & Hijmans, 2017; Trabucco & Zomer, 2010) soil–water balance layers, using ArcMap 10.5 (ArcGIS Desktop, ESRI, CA, USA). To account for within-site climatic heterogeneity, a 2 km buffer zone around each population sampling location was used to extract climatic data.

At each population, we established a 20 × 20 m plot and collected mature seeds and fresh leaves from 20 maternal plants separated by at least 3 meters from each other to avoid sampling closely related individuals. All seeds from fully developed fruits were collected from each maternal plant. Leaves and seeds from each maternal plant were stored separately in paper bags at room temperature until DNA extraction and the beginning of our common garden experiment, respectively (see below). To characterize soil properties, we also collected three soil cores (0–20 cm depth) at the centre and two random corners of each plot, and stored them individually. From soil samples, we determined total S (as a proxy of gypsum content), C, and N concentrations, using an elemental analyser (TruSpec CHNS, LECO, MI, USA), organic matter content, estimated by chromic acid digestion, and available Olsen P, using standardized protocols with $NaHCO_3$ (Olsen et al., 1954) of each population. Soil samples were analysed at IPE-CSIC (Zaragoza, Spain). Fieldwork did not need any permission.

2.2 | Molecular analyses

To assess population differentiation in molecular markers (F_{ST}), we first extracted genomic DNA from air-dried field-collected leaves, using a commercial kit (Dneasy Plant Minikit; QIAGEN, Germany). Then, individuals were genotyped using 10 species-specific nuclear polymorphic microsatellite markers (Martínez-Nieto

TABLE 1 Location, geographical coordinates, altitude, climatic conditions, and soil gypsum content of the 11 sampled populations of *Lepidium subulatum* L.

Population code	Population location (province)	Geographical coordinates (WGS84)		Altitude (m asl)	T. mean (°C)	T. min. (°C)	T. max. (°C)	Prec. (mm)	Gypsum content (%)
BAL	Los Balbases (Burgos)	42° 13′ 20.3″ N	4° 4′ 30.9″ W	851	11.47	4.36	19.52	460.2	56.67
PEÑ	Peñafiel (Valladolid)	41° 35′ 25.0″ N	4° 6′ 30.1″ W	815	11.97	4.46	20.37	386.5	85.69
ALF	Alfajarín (Zaragoza)	41° 37′ 25.5″ N	0° 41′ 52.3″ W	219	15.17	7.46	23.59	371.8	52.00
GEL	Gelsa (Zaragoza)	41° 27′ 5.3″ N	0° 22′ 24.6″ W	254	15.36	7.42	24.01	372.9	76.22
SPP	San Pedro Palmiches (Cuenca)	40° 25′ 51.9″ N	2° 23′ 51.1″ W	850	13.50	5.75	22.60	450.3	87.92
PDG	Portalrubio de Guadamejud (Cuenca)	40° 16′ 15.8″ N	2° 35′ 14.7″ W	794	13.54	5.68	22.77	448.8	26.21
SMV	San Martín de la Vega (Madrid)	40° 13′ 19.2″ N	3° 35′ 3.3″ W	551	14.73	6.76	23.83	393.9	67.84
CAB	Cabezo Redondo (Alicante)	38° 38′ 32.9″ N	0° 53′ 33.5″ W	533	15.13	7.62	23.69	358.0	76.57
ECZ	Escúzar (Granada)	37° 3′ 20.2″ N	3° 44′ 41.5″ W	927	14.78	7.42	23.37	462.3	80.22
TOP	Topares (Almería)	37° 52′ 18.4″ N	2° 11′ 22.0″ W	1157	12.40	4.26	22.10	451.6	86.93
VY	Venta de Yesos (Almería)	37° 5′ 2.3″ N	2° 17′ 7.3″ W	539	15.95	8.67	24.36	303.0	80.98

Note: Climatic data were extracted from WorldClim bioclimatic layers using a 2 km buffer zone. T. mean: annual mean temperature (bio1); T. min.: mean temperature of coldest quarter (bio11); T. max.: mean temperature of warmest quarter (bio10); Prec.: annual precipitation (bio12). Detailed climatic data and soil composition in each population and CULTIVE facilities can be found in Table S1.

et al., 2012). Detailed information about DNA extractions, PCR reactions, and microsatellite genotyping can be found in Blanco-Sánchez et al. (2021).

2.3 | Set-up of common garden experiment

We established a common garden experiment in the CULTIVE facilities at URJC (Móstoles, Madrid, Spain). Before sowing, 10 maternal plants per population were randomly selected, and 10 seeds per plant were individually weighed using a Mettler Toledo MX5 microbalance (1 µg precision; Mettler Toledo, Columbus, OH, USA) to obtain a family-level seed mass. In mid-July 2018, seeds from each maternal plant and population were sown in 0.5 L pots (Alpifer, Valencia, Spain) filled with soil extracted from a gypsum quarry close to the experimental site (Yesos Ibéricos-Algiss S.A., Valdemoro, Madrid, Spain). This soil was analysed in the same way as the soil collected at the studied populations (Table S1). Since *L. subulatum* pollination is mostly outcrossing and different flowers are often pollinated at varying times by a diversity of insects (and consequently, by different paternal individuals), individuals from the same maternal plant constituted a maternal family and were considered half-siblings. To maximize seed germination and establishment, pots were placed in a greenhouse and maintained in well-watered conditions (i.e. at field capacity) for ~3 months. To confirm that the substrate did not contain seeds of *L. subulatum*, control pots were also filled and placed in the greenhouse. The control pots did not show any germination from the seed bank. In October 2018, seedlings were individually transplanted into 6 L pots (22 × 20 cm; Alpifer, Valencia, Spain) filled with the same substrate, and moved to the outdoors cultivation facility. Experimental individuals were grown in common, optimum conditions until the implementation of watering treatments.

To minimize potential maternal effects, which are expected to be greater in early phases of the life cycle (Auge et al., 2017; Bischoff & Müller-Schärer, 2010), and because most individuals of *L. subulatum* reach the reproductive stage in their second growing season, watering treatments were applied after two growing seasons. The watering treatments lasted for ~3 months, after which we characterized the phenotype of experimental individuals. In late-February 2020, five plants of each maternal family and population were randomly assigned to each of two contrasting watering treatments, well-watered and drought ($N = 1100$ plants; 11 populations × 10 families/population × 5 individuals/family/treatment × 2 watering treatments). To implement our watering treatments, pots were placed under six rain exclusion structures (three per treatment) that eliminated natural precipitation without substantially affecting other environmental conditions (see details in Methods S1). Specifically, PAR values exceeded 1800 $\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ in full-sun days. Treatments were implemented using a drip-irrigation system with pressure-compensating emitters (Rain Bird XB05PC; Rain Bird Corporation, CA, USA), adjusting the number and duration of watering events to reach ecologically meaningful levels of soil water content (SWC

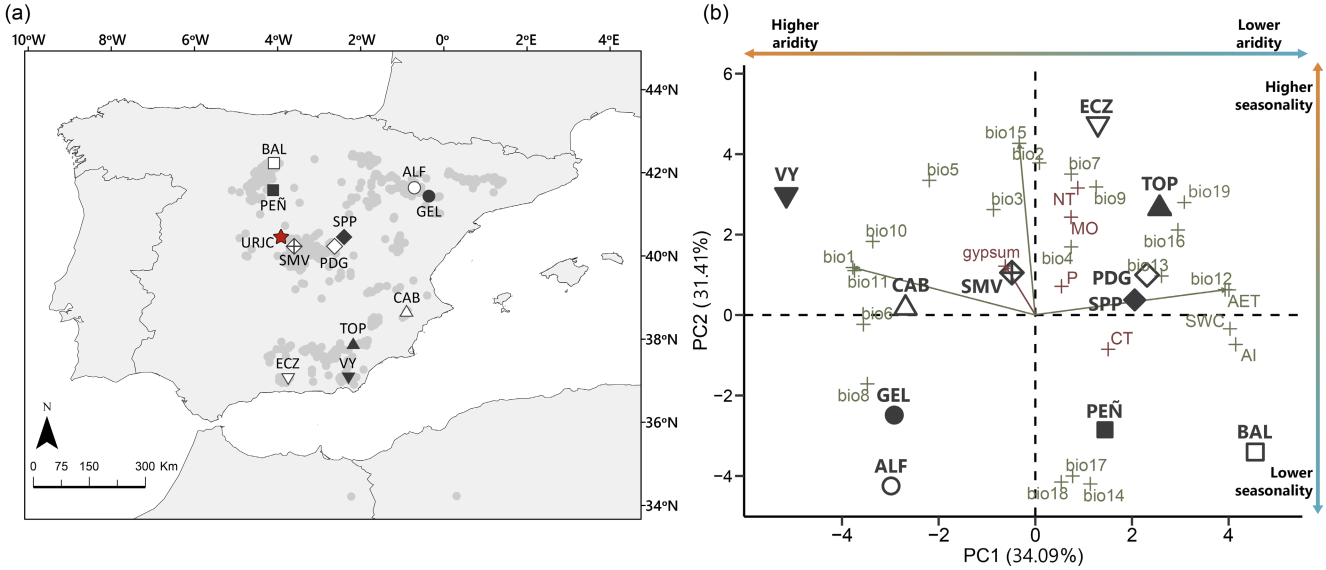

FIGURE 1 (a) Map of the Iberian Peninsula showing sampled populations. Grey-coloured area indicates the worldwide distribution of *Lepidium subulatum* extracted from GBIF records. Each studied population is represented with a different symbol, and population codes are those in Table 1. The location of CULTIVE facilities (URJC), where the common garden experiment was performed, is shown with a red star symbol; (b) Principal component analysis (PCA) used to summarize environmental variables (climate and soil composition) of populations. The proportion of variance explained by the two first PCA axes is shown in parenthesis (see PC1 vs. PC3 in Figure S4). Loadings and names of climatic and soil composition variables are shown in green and maroon, respectively. Bio1: annual mean temperature; bio2: mean diurnal range; bio3: isothermality; bio4: temperature seasonality; bio5: maximum temperature of warmest month; bio6: minimum temperature of coldest month; bio7: temperature annual range; bio8: mean temperature of wettest quarter; bio9: mean temperature of driest quarter; bio10: mean temperature of warmest quarter; bio11: mean temperature of coldest quarter; bio12: annual precipitation; bio13: precipitation of wettest month; bio14: precipitation of driest month; bio15: precipitation seasonality; bio16: precipitation of wettest quarter; bio17: precipitation of driest quarter; bio18: precipitation of warmest quarter; bio19: precipitation of coldest quarter; SWC: soil water content; AI: aridity index; AET: actual evapotranspiration; CT: soil total Carbon; NT: soil total Nitrogen; P: available Olsen Phosphorous; OM: organic matter (%); gypsum: soil gypsum content (%).

hereafter) in the experimental pots, that simulated the spatiotemporal heterogeneity of gypsum habitats (Blanco-Sánchez et al., 2022). In the well-watered treatment, plants were kept at field capacity (~25% of SWC for our substrate), simulating conditions experienced in climatically milder populations or periods when soil moisture is high (e.g. in early spring). In contrast, SWC in the drought treatment was gradually reduced and then maintained at ~50% of field capacity (12%–14% of SWC), simulating the climatic conditions of populations with harsher conditions or periods when soil moisture is lower (e.g. in early summer). During the experiment, we monitored SWC of 30 pots per treatment (10 per rain exclusion structure) every 2–4 days, using an HH2 Moisture Meter with an ML3 Sensor (Delta-T devices, Cambridge, UK; see Figure S1). The watering treatments were maintained for ~3 months, ending when plants in the well-watered treatment showed senescent leaves (June 2020).

2.4 | Phenotypic and fitness characterization of populations

We measured a suite of traits related to resource-use, growth, phenology, and reproductive fitness. Specifically, we measured the height, maximum diameter, and orthogonal diameter to the maximum diameter in all plants at the onset and the end of the watering treatments. From these, we calculated initial and final plant volume as the volume of a hemispheroid, $\frac{2}{3}\,\pi\, r_1\, r_2\, h$, where r_1 is the maximum radius, r_2 is the perpendicular radius to maximum radius and h is the height of the plant; and volume-based relative growth rate (RGR) as $\frac{(\ln V_2 - \ln V_1)}{T_{2\text{-}1}}$, where V_1 and V_2 are initial and final measurements of plant volume, respectively, and $T_{2\text{-}1}$ is the time elapsed between the two measurements (≈100 days).

During the experiment, we monitored the reproductive phenology of all plants approximately every 3 days (26 censuses). Following the phenological events described in Palacio and Montserrat-Martí (2005), we recorded the onset of flower bud formation, open flowers and fully developed fruits for each plant, and the proportion of reproductive individuals in each census. At harvest, we visually estimated the percentage of senescent leaves in each plant. Phenological censuses were always performed by the same researcher.

In late-May 2020, we randomly collected eight non-senescent leaves per plant, storing them in zipper plastic bags with moisturized filter papers. Leaves were rehydrated for 12 h, scanned using an Epson Perfection V370 Photo scanner (Seiko Epson Corporation, Japan), and oven-dried at 60°C for 48 h. Dried leaves were weighed using a microbalance, and leaf area (LA) and specific leaf area (SLA hereafter; the one-sided area of water-saturated leaves divided by their oven-dry mass) were calculated. Using these leaves, we also determined leaf carbon and nitrogen content, and carbon and nitrogen stable isotope ratios (δ^{13}C and δ^{15}N; a proxy of water use efficiency and N assimilation, respectively) in three half-siblings per population, maternal family, and treatment ($N = 660$). These analyses were conducted at UC Davis Stable Isotope Facility (Davis, CA, USA). We also measured the midday maximum photochemical efficiency (F_v/F_m), using a Handy PEA+ chlorophyll fluorimeter (Hansatech, UK) during three consecutive full-sun days (from 13:00 to 17:30, UTC+2). Measurements were taken after adapting leaves to the dark for 30 min.

Before harvesting, we counted the inflorescences in all plants, and when possible, measured the length of three randomly selected inflorescences. Reproductive biomass (i.e. all the inflorescences produced by each individual) was collected, weighed in a microbalance, and thoroughly cleaned to separate viable seeds. Ten viable seeds per plant were individually weighed using a microbalance. Finally, above-ground tissues were harvested, oven-dried and weighed in a Kern ABJ 120-4M analytical balance (1 mg precision; Kern & Sohn GmbH, Germany).

2.5 | Statistical analyses

2.5.1 | Population differentiation in neutral markers, quantitative traits, and plasticity patterns

To assess genetically based differences among populations in quantitative traits, the effect of watering treatments on trait expression (i.e. phenotypic plasticity) and to what extent populations differed in their plastic responses (i.e. population by environment interaction, or P×T), we fitted linear mixed models with restricted maximum likelihood (REML), including individual trait values as the dependent variable, population, treatment and the population-by-treatment interaction as fixed factors, and maternal family as a random factor. Furthermore, to consider potential factors affecting trait expression, family-level seed mass, and the identity of rain exclusion structures were included as fixed covariates. The significance of fixed factors was assessed using function *Anova* (package car; Fox et al., 2012) with type III sum of squares and the Kenward–Roger approach. Marginal and conditional R^2 (i.e. the proportion of variance explained by fixed factors, and by all factors in the models, respectively), were calculated for each model using function *r.squaredGLMM* (package MuMIn; Barton, 2020). To avoid issues caused by multiple testing, *p*-values for each term were corrected by false discovery rate (Benjamini & Hochberg, 1995) using function *p.adjust*. A significant effect of population indicated genetically based differences among populations in quantitative traits; a significant effect of treatment indicated significant phenotypic differences across treatments (phenotypic plasticity); and a significant population-by-treatment interaction indicated differences in plasticity among populations (i.e. differential plasticity; P×T).

Then, we calculated quantitative genetic differentiation among populations for each trait within each treatment (Q_{ST}) by partitioning the total genetic variance into the between- and within-population components (σ_B^2 and σ_W^2, respectively), using the following formula (Spitze, 1993):

$$Q_{ST} = \frac{\sigma_B^2}{\left(\sigma_B^2 + 2 \cdot \sigma_W^2\right)} = \frac{V_P}{V_P + 2 \cdot (4 \cdot V_F)}$$

Variance components for the calculation of mean Q_{ST} and Q_{ST} distributions for each trait and treatment were estimated using Bayesian mixed models with MCMCglmm package (Hadfield et al., 2019). We ran 5,000,000 iterations of Markov chain Monte Carlo (MCMC), with a burning period of 500,000 and a thinning interval of 5000 iterations, and non-informative inverse Wishart priors were set ($V = 1$, $nu = 0.002$; Hadfield, 2010). Population and maternal family were included in the models as random factors. Within-population variance component $\left(\sigma_W^2\right)$ was calculated by multiplying the variance among families (V_F) by four, because individuals from the same family were considered half-siblings (Ramírez-Valiente et al., 2018; Whitlock & Guillaume, 2009). To minimize potential maternal effects and environmental differences across rain exclusion structures affecting the phenotypic expression of individuals, family-level seed mass and the identity of rain exclusion structures were also included in the models as fixed covariates.

To assess population differentiation in molecular markers, we calculated the F_{ST} distribution from microsatellite data. Briefly, we generated 10,000 bootstrapped F_{ST} values across loci using GDA 1.1 (Lewis & Zaykin, 2002), and these values were multiplied by Lewontin–Krakauer χ^2 distribution (Lewontin & Krakauer, 1973) to account for potential deviations in F_{ST} among loci caused by demographic factors (Whitlock, 2008; Whitlock & Guillaume, 2009; see similar approaches in Hernández-Serrano et al., 2014; Ramírez-Valiente et al., 2018).

Finally, we also assessed whether population differentiation in molecular markers was related to geographic distance (isolation by distance; IBD) and environmental differences between populations (isolation by environment; IBE), which could act as confounding factors to determine the relationship between phenotypic variation and environmental differences across populations. Specifically, we performed a Mantel correlogram (Legendre & Legendre, 2012) between the pairwise genetic distance matrix (pairwise F_{ST}) and (a) the pairwise geographical distance matrix (Euclidean distance), and (b) the pairwise environmental distance matrix. The environmental distance matrix was calculated using the four environmental variables selected for univariate phenotype–environment associations (see below). Geographical and environmental distances were assessed using function *distance* (package ecodist; Goslee & Urban, 2007), and Mantel correlograms were performed using function *mantel.correlog* (package vegan; Oksanen et al., 2019). The size and the number of distance classes was assessed using Sturge's rule (Legendre & Legendre, 2012), and the significance of correlations was tested after 99,999 permutations and corrected by false discovery rate.

2.6 | $Q_{ST} - F_{ST}$ Comparisons

To look for evidence of past natural selection, we compared the Q_{ST} distribution for each trait in each treatment with the F_{ST} distribution inferred from microsatellites. F_{ST} (Wright, 1951) quantifies population differentiation in molecular markers, caused by neutral evolutionary processes (Leinonen et al., 2013; Whitlock, 2008). Analogous to F_{ST}, Q_{ST} (Spitze, 1993) measures population genetic differentiation in phenotypic traits. When $Q_{ST} > F_{ST}$, trait divergence among populations is significantly higher than expected by neutral processes, which suggests that past natural selection has favoured different genetically based phenotypes in different populations (spatially divergent selection). In contrast, when $Q_{ST} < F_{ST}$, phenotypic differentiation is significantly lower than expected by neutral processes, suggesting that similar phenotypes have been favoured across populations (spatially homogenizing selection). Finally, when $Q_{ST} \approx F_{ST}$, there is no evidence that population differentiation was caused by anything other than neutral processes (Leinonen et al., 2013; Merilä & Crnokrak, 2001; Whitlock, 2008). Since F_{ST} estimates are extremely variable across loci, Whitlock (2008) recommended that the distributions of Q_{ST} and F_{ST}, rather than their mean values, should be compared to interpret the results obtained from $Q_{ST} - F_{ST}$ comparisons. Therefore, we first compared the 95% CIs of both parameters, and considered that Q_{ST} for a particular trait was not statistically different from F_{ST} when their CIs overlapped (Marin et al., 2020). Then, we compared the distributions of Q_{ST} and F_{ST} using Kruskal–Wallis nonparametric tests (see also Ramírez-Valiente et al., 2018).

2.7 | Phenotype–environment and plasticity–environment associations

To test whether population differentiation in quantitative traits was associated with local climatic conditions or the soil characteristics of populations, we performed both univariate and multivariate associations between: (a) populations' trait means in each treatment, and (b) their plasticity, with the climatic conditions and soil chemical composition of populations. Significant univariate and multivariate phenotype–environment and plasticity–environment associations would indicate the presence of clinal variation in traits and their plasticity along environmental gradients.

First, to obtain treatment-specific populations' trait means, we fitted within-treatment linear mixed models with individual trait values as the dependent variable, population, family-level seed mass, and rain exclusion structure as fixed factors, and maternal family as a random factor. Populations' trait means for each watering treatment were extracted from these models using emmeans package (Lenth, 2021). Furthermore, for traits that showed significant variation for plasticity among populations (i.e. P × T), we calculated a plasticity index (relative distances plasticity index; RDPI) for each population by calculating the mean of the RDPIs of each maternal family within each population. This index assesses the mean relative phenotypic distance for each trait between all pairs of individuals of the same maternal family grown in different environmental conditions (Valladares et al., 2006). To incorporate the diversity of norms of reaction in each population, RDPIs at the population level were assessed by calculating the mean RDPI of the different families from each population, as recommended (Valladares et al., 2006), that is, at the lowest level of genetic replication in our experimental design.

For univariate associations, we selected four environmental variables that showed the highest loadings across the three main axes of the PCA used to summarize the climatic conditions and soil chemical composition of populations (environmental PCA hereafter; see Figure 1b and Section 3): mean annual temperature and annual precipitation (bio1 and bio12 from WorldClim layers, respectively; PC1), precipitation seasonality (bio15; PC2) and soil gypsum content (PC3). First, we assessed the presence of a geographical pattern in the selected environmental variables, which could affect the interpretation of the phenotype–environment associations. To do so, we performed: (i) Pearson's pairwise correlations between the environmental variables and the geographical location (latitude and longitude) of populations; and (ii) a Mantel correlogram between the pairwise geographical distance matrix and the pairwise environmental distance matrix. Importantly, we did not find a geographic pattern in the main selective pressures associated with adaptive differentiation (mean temperature and annual precipitation; see Results section) in our study populations (see results in Tables S2 and S3). Finally, we explored univariate associations between populations' trait means in each treatment and trait plasticity with the local climate, latitude, and soil chemical composition of populations by calculating pairwise Pearson correlations.

Furthermore, we corroborated the results from the phenotype–environment associations while controlling for the potential effect of the neutral genetic structure and spatial structure of populations on phenotypic differentiation. Specifically, we performed partial Mantel tests between the pairwise phenotypic distance matrices in each treatment and the pairwise environmental distance matrix, including (i) the pairwise geographical matrix, and (ii) the environmental distance matrix as a covariate. Partial Mantel tests were performed using function *mantel* (package vegan; Oksanen et al., 2019), and the significance of correlations was tested after 99,999 permutations and corrected by using false discovery rate.

We also assessed whether: (a) multivariate phenotypic differentiation of populations in each watering treatment and (b) multivariate plasticity were associated with multivariate differences among populations in climatic conditions and soil chemical composition. First, we summarized the multivariate phenotypic differences across populations in quantitative traits performing two PCAs, one per treatment, using the population means of all traits (phenotypic PCA in well-watered and drought, hereafter). Then, multivariate plasticity was calculated by performing one PCA including populations' trait means in both watering treatments, which allowed us to draw a vector of phenotypic change that connected the multivariate phenotype of each population in each treatment in a multivariate phenotypic space (i.e., a multivariate reaction norm for each population; Collyer & Adams, 2007; Solé-Medina et al., 2022). From the two first axes of this PCA, we calculated the magnitude and direction of the multivariate plasticity vector for each population. Differences between populations in the length of this vector indicated differences in the magnitude of plasticity, while differences in direction showed a shift in the traits involved in the multivariate phenotypic change. All variables were scaled and centred before performing all PCAs. Finally, we explored multivariate associations between (a) the axes (first and second) of the well-watered and drought phenotypic PCA performed to assess multivariate quantitative genetic differentiation in each treatment and (b) vector length and vector direction of multivariate plasticity for each population, with the three first axes of the environmental PCA used to summarize multivariate environmental differences among populations, by calculating pairwise Pearson correlations.

To avoid issues related to multiple testing, *p-values* of both univariate and multivariate associations were corrected using false discovery rate. We performed all analyses using R v4.0.5 (R Core Team, 2018).

3 | RESULTS

3.1 | Genetic differentiation among populations and phenotypic plasticity patterns

We detected genetically based phenotypic differences among populations in all the traits evaluated (significant effect of Population; Table 2), showing evidence of significant population differentiation. In addition, we also found significant differences in the phenotypic expression of populations between treatments (i.e. phenotypic plasticity, significant effect of Treatment; Table 2). Specifically, plants showed an overall ~50% reduction in their volume and RGR, and 30% less above-ground biomass in response to drought. Individuals also showed significant changes in leaf morphology, with an overall increase of ~9% in SLA and a ~33% decrease in LA in the drought treatment. Physiological and leaf chemical composition traits were also affected by drought. On average, plants in the drought treatment

TABLE 2 Results from linear mixed models testing the effect of population (df = 10), treatment (i.e. phenotypic plasticity; df = 1) and their interaction (P × T; df = 10) in the phenotypic expression.

	Population		Treatment		Population × treatment			
	F/X^2	p	F/X^2	p	F/X^2	p	R^2_M	R^2_C
F_v/F_m (FvFm)	6.143	**<0.001**	95.178	**<0.001**	1.031	0.414	0.144	0.150
δ^{13}C (d13C)	5.589	**<0.001**	148.935	**<0.001**	2.454	**0.039**	0.280	0.361
δ^{15}N (d15N)	4.567	**<0.001**	257.602	**<0.001**	1.945	0.116	0.340	0.346
Leaf C content (C)	3.157	**0.002**	139.380	**<0.001**	7.508	**<0.001**	0.289	0.314
Leaf N content (N)	19.805	**<0.001**	0.008	0.931	3.717	**<0.001**	0.334	0.357
Leaf area (LA)	40.461	**<0.001**	420.358	**<0.001**	0.979	0.460	0.503	0.521
SLA (SLA)	106.895	**<0.001**	109.245	**<0.001**	1.362	0.193	0.608	0.624
Initial plant volume (Initial_PV)	30.961	**<0.001**	0.030	0.863	1.025	0.420	0.330	0.361
Final plant volume (Final_PV)	41.063	**<0.001**	189.742	**<0.001**	2.318	**0.040**	0.457	0.488
Relative growth rate (RGR)	4.390	**<0.001**	300.916	**<0.001**	0.482	0.902	0.274	0.274
Aerial biomass (AB)	12.981	**<0.001**	99.789	**<0.001**	1.264	0.246	0.255	0.281
Flower bud onset (FBF)	33.290	**<0.001**	1.178	0.278	0.296	0.982	0.371	0.399
Flowering onset (FlO)	26.092	**<0.001**	0.134	0.715	0.758	0.669	0.354	0.401
Fruiting onset (FrO)	39.860	**<0.001**	3.041	0.109	0.404	0.945	0.434	0.462
Proportion of reproductive plants (Prop_F)	113.854	**<0.001**	0.032	0.859	5.959	0.819	0.690	0.709
Senescence (Sen)	7.129	**<0.001**	426.913	**<0.001**	1.580	0.107	0.330	0.363
Reproductive biomass (Inf_Mass)	20.045	**<0.001**	28.763	**<0.001**	1.081	0.374	0.280	0.292
Number of inflorescences (Inf_Number)	291.428	**<0.001**	3.789	*0.074*	87.441	**<0.001**	0.799	0.965
Inflorescence size (Inf_Size)	17.950	**<0.001**	239.970	**<0.001**	1.685	0.220	0.478	0.504
Individual seed mass (Seed_Mass)	15.070	**<0.001**	25.638	**<0.001**	2.351	**0.040**	0.562	0.591

Note: Models included maternal seed size (df = 1) and rain exclusion structure (df = 2) as covariates (see their effects in Table S4) and maternal family as a random factor. *F*-statistics (X^2 for the generalized mixed models performed for italicized traits), *p*-values, marginal, and conditional variance (R^2_M and R^2_C, respectively) for each model are shown. Significant ($p < 0.05$) and marginally significant ($0.05 < p < 0.1$) terms after false discovery rate correction are presented in bold and italic, respectively. Abbreviations of traits shown in Figure 4 are indicated in parentheses.

showed higher $\delta^{13}C$ (i.e. water use efficiency; WUE hereafter) and leaf C (increase of ~2.7% and 2%, respectively), and lower photochemical efficiency and $\delta^{15}N$ (reduction of ~10.6% and 30%, respectively). Fitness traits were also influenced by the watering treatments, with higher reproductive biomass and inflorescence size (~50% increase) and lower individual seed mass (~7.8%) under well-watered conditions.

Plastic responses were generally similar across populations (i.e. no P×T, parallel norms of reaction; Table 2, Figure S2). However, populations expressed significant differential plasticity in a few traits ($\delta^{13}C$, leaf C and N content, final plant volume, number of inflorescences and individual seed mass; Table 2). Conditional R^2 was higher than marginal R^2 in the models of all traits except RGR, indicating substantial variation across families within populations. The effect of maternal seed size was not significant for most functional traits (Table S4).

3.2 | Univariate phenotype–environment and plasticity–environment associations

We found significant univariate associations between quantitative genetic differences among populations in both treatments (extracted from the within-treatment models) and the local climate of populations (extracted from WorldClim bioclimatic layers; Figure 2; Figure S3), that is, clinal variation in quantitative traits along environmental gradients. Importantly, significant associations between phenotypic and environmental distances of populations were also detected when the geographic and the genetic distances among populations were considered as covariates, that is, when the potential effect of non-adaptive neutral processes on phenotypic differentiation was controlled (see results from partial Mantel tests in Table S5).

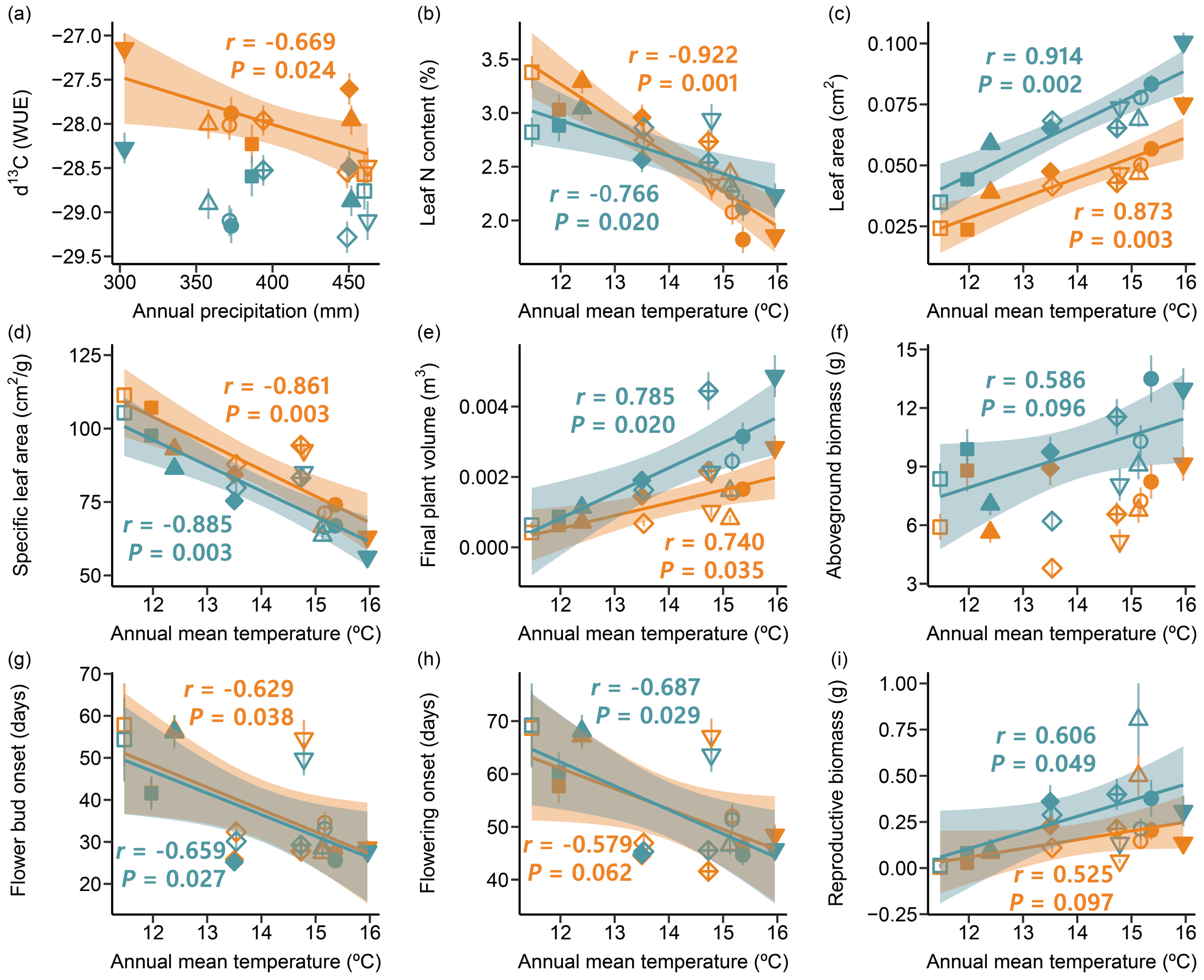

FIGURE 2 Univariate correlations between annual mean temperature and annual precipitation and populations' trait means for (a) δ13C, (b) leaf N content, (c) leaf area, (d) SLA, (e) final plant volume, (f) above-ground biomass, (g) flower bud onset, (h) flowering onset, and (i) reproductive biomass, in our common garden experiment (well-watered and drought treatment in blue and orange, respectively). Populations' trait means and SE are shown, using a different symbol for each population (matching those in Figure 1). Pearson correlation results (*r* and *p*) and regression lines for significant and marginally significant relationships are shown. Shaded areas represent 95% CIs.

In both watering treatments, individuals from warmer populations (higher annual mean temperature) had significantly greater LA and both initial and final plant volume, but lower SLA and leaf N content (i.e., more sclerophyllous leaves) than individuals from colder populations (Figure 2). Annual mean temperature was also negatively associated with phenology and positively associated with the length and biomass of inflorescences in both treatments, especially under well-watered conditions, indicating that individuals from warmer populations flowered significantly earlier and showed higher reproductive fitness (Figure 2). Furthermore, we found a negative association between δ^{13}C and annual mean precipitation of populations (only in the drought treatment), indicating that populations from drier sites showed higher WUE under drought conditions (Figure 2). In contrast, neither precipitation seasonality, latitude, nor soil composition of the sampled populations were associated with populations' trait means in either treatment (Figure S3).

Finally, for several traits showing population differentiation in plasticity (i.e. significant P×T), we also found significant univariate associations between the magnitude of plasticity and their local climate. Differences in plasticity of WUE and leaf N content were positively associated with annual mean temperature and negatively associated with annual precipitation, indicating that more arid populations expressed higher plasticity in δ^{13}C and leaf N (Figure 3).

3.3 | Multivariate phenotype–environment and plasticity–environment associations

We found significant associations between the multivariate environmental PCA and the multivariate phenotypic PCAs summarizing quantitative genetic differentiation among populations in both treatments (Figures 1b and 4; see also Figure S4). The first axis of the environmental PCA (Figure 1b) explained 34.09% of the variance and was positively associated with mean temperature and negatively associated with mean precipitation. The second axis explained 31.41% of the environmental variance and was positively related to the climatic seasonality of populations. Accordingly, populations with higher and lower eigenvalues of PC1 and PC2, respectively, experience lower climatic stress (i.e., higher precipitation, and lower temperature and seasonality; Figure 1b). Finally, the third axis of the environmental PCA explained 14.38% of the variance and was positively associated with soil total N, C, P, and organic matter, and negatively associated with the gypsum content of populations (Figure S4).

The phenotypic PCAs from both treatments showed similar trait loadings and similar variance explained (Figure 4). PC1 in both the well-watered and the drought treatment phenotypic PCAs explained 44.35% and 45.32% of the variance, respectively, and were positively associated with leaf N, δ^{15}N, phenology, and SLA; and negatively with plant size (above-ground biomass, initial and final plant volume), fitness (length and biomass of inflorescences), senescence, δ^{13}C, and LA. PC2 explained 17.16% (well-watered) and 17.87% (drought) of the variance, and showed positive loadings for leaf C content, the number of inflorescences and the proportion of reproductive plants; and negative for F_v/F_m in both treatments.

We found significant associations between PC1 of the environmental PCA and PC1 of both phenotypic PCAs (Figure 4; Figure S5). These results indicated that populations from areas with harsher (i.e. warmer and drier) conditions showed larger but more sclerophyllous leaves (i.e. higher leaf area and lower SLA) with lower N content, higher WUE, above-ground biomass and fitness (greater length and mass of inflorescences), and earlier

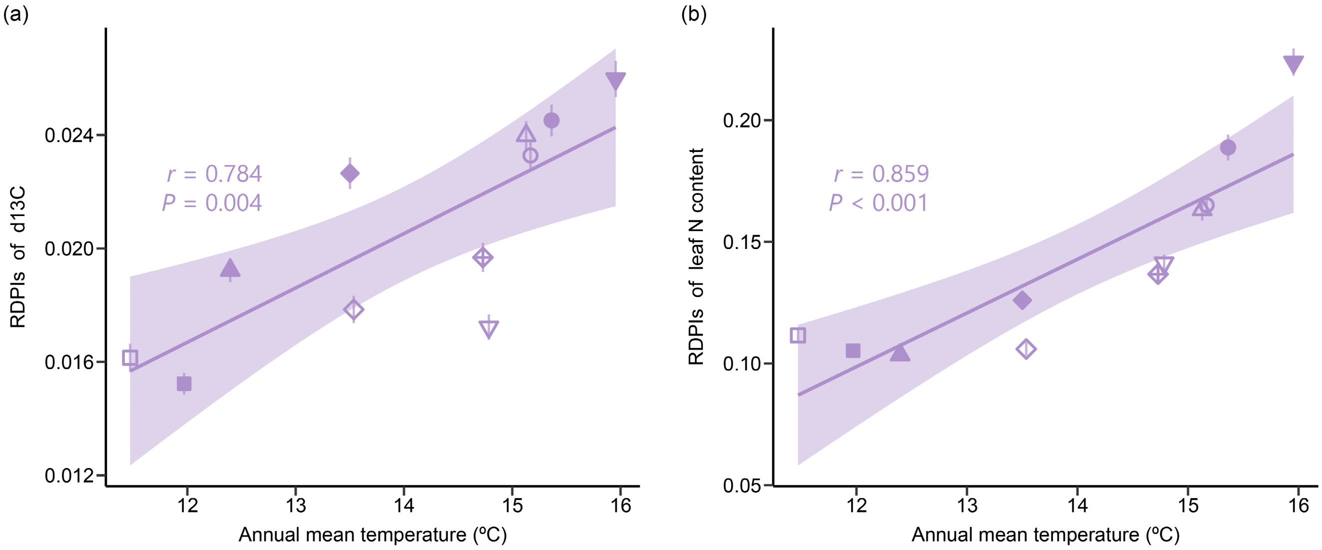

FIGURE 3 Univariate correlations between annual mean temperature and phenotypic plasticity means (RDPIs) of populations for (a) δ^{13}C, and (b) leaf N content. Means of plasticity and SE are shown using a different symbol for each population (matching those in Figure 1). Pearson correlation results (*r* and *p*) and regression lines are shown. Shaded areas represent 95% CIs.

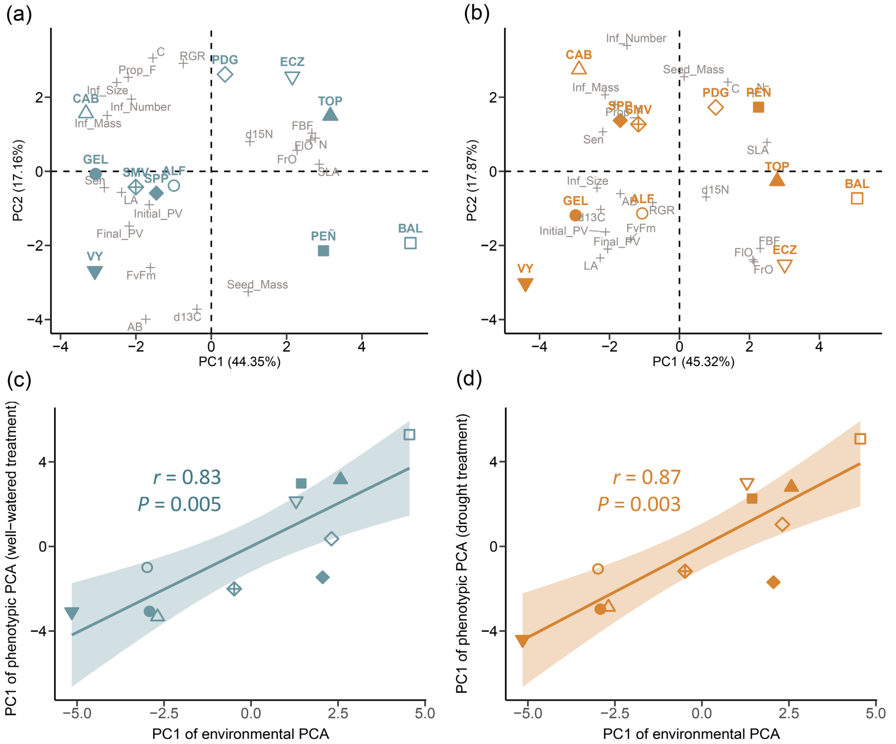

FIGURE 4 Principal component analysis (PCA) used to summarize quantitative trait expression of populations in the (a) well-watered and (b) the drought treatment; and multivariate associations between summarized climate (PC1) and summarized quantitative variation of populations (PC1) in the (c) well-watered and (d) the drought treatment. Each population is represented with a different symbol (matching those in Figure 1), and population codes are those in Table 1. In the PCA, the proportion of variance explained by the two first PCA axes is shown in parenthesis. Loadings and names of functional traits are shown in grey. Abbreviations of traits can be found in Table 2. Multivariate associations show Pearson correlation results (*r* and *p*) after false discovery rate correction, and regression lines for each treatment, with shaded areas representing 95% CIs.

phenologies than those from populations with milder climatic conditions. Conversely, there were not any significant correlations between any other environmental and phenotypic PCs ($p > 0.05$ in all cases; Figure S5).

Finally, neither the magnitude nor the direction of multivariate plasticity was significantly correlated with either of the three first axes of the environmental PCA ($p > 0.05$ in all cases; Figure S5). Both the magnitude and the direction of multivariate plasticity were very similar across populations (i.e. parallel multivariate reaction norms; Figure S6), supporting the lack of P×T found in the plasticity patterns of individual traits (Table 2; Figure S2).

3.4 | F_{ST} – Q_{ST} comparisons

Genetic differentiation in microsatellites (F_{ST}) was significantly greater than zero (posterior mean = 0.187; CIs = 0.145–0.224; Figure 5). Genetic differentiation in molecular markers was neither influenced by the geographic distance nor by the environmental differences between populations, supporting the neutrality of these markers (see results from Mantel correlogram and Mantel test; Table S6). We found significant quantitative genetic differentiation across populations (Q_{ST}) for morphological, physiological, phenological, and fitness traits in both treatments. In all cases, Kruskal–Wallis tests showed that Q_{ST} distributions were significantly higher than the F_{ST} distribution (Figure 5). Specifically, Q_{ST} distributions were significantly higher than the F_{ST} distribution for SLA, initial and final plant volume, RGR, onset of flower bud, flowering and fruiting, and reproductive biomass in both treatments, for above-ground biomass, senescence, number of inflorescences and inflorescences size in the well-watered treatment, and for leaf N in the drought treatment (Figure 5).

4 | DISCUSSION

Our study characterized phenotypic differentiation among populations across the environmental range of the Mediterranean gypsum endemic shrub *Lepidium subulatum*, and investigated the importance of plasticity and past natural selection in determining population differentiation using a common garden experiment. We found multiple lines of evidence suggesting that climate-driven selection has played a key role in shaping trait differences among populations. In both watering treatments, population differentiation in morphological, physiological, phenological, and fitness traits was significantly higher than differentiation in neutral molecular markers. Furthermore, clinal variation in ecophysiological traits was associated with annual temperature and precipitation even after accounting for the potential effect of neutral processes on phenotypic differentiation, but not with differences in soil composition. Phenotypic plasticity patterns were generally similar across populations, but we found differential plasticity associated with populations' climate in two key physiological traits. Overall, our results show genetically based intraspecific differentiation in ecophysiological and fitness traits that is consistent with divergent evolution under contrasting climates, which suggests that habitat specialization has not constrained adaptation to climate of populations in *Lepidium subulatum*.

Widespread Mediterranean gypsophiles experience edaphically and climatically stressful conditions that substantially vary across

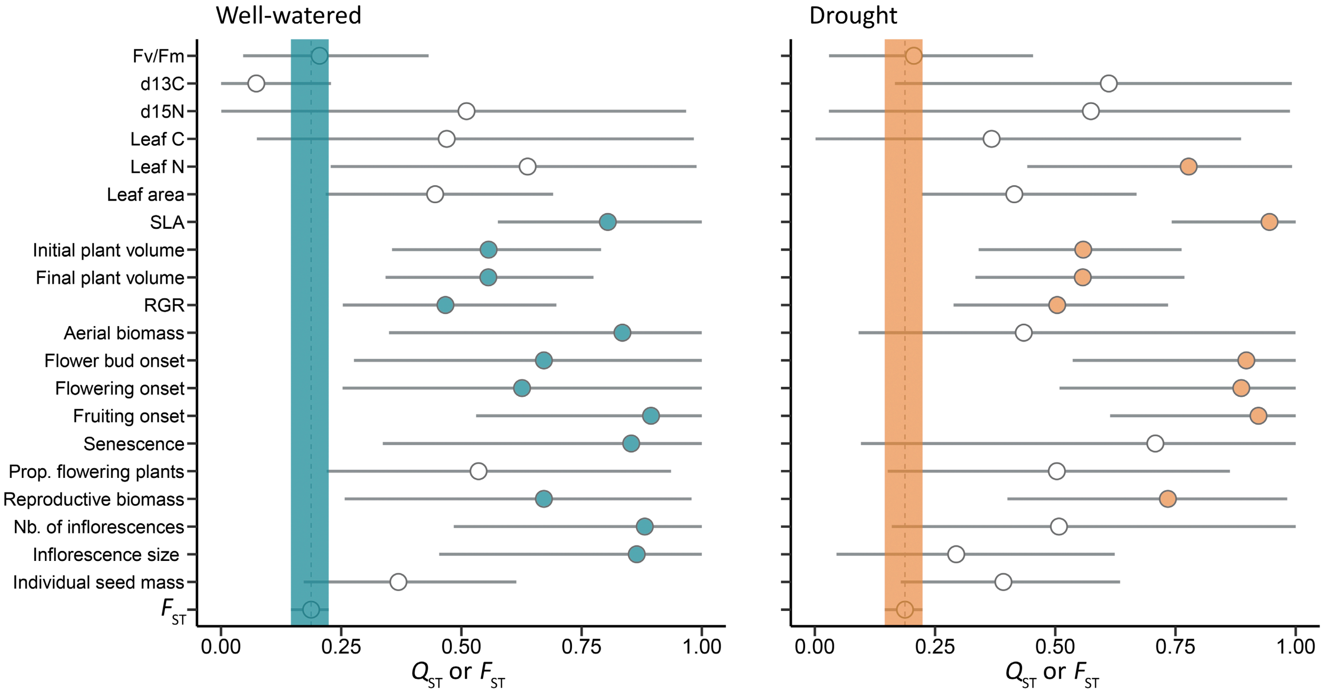

FIGURE 5 F_{ST} and Q_{ST} posterior means and 95% CIs for all phenotypic traits measured in 11 populations of *Lepidium subulatum* in the common garden experiment under well-watered and drought conditions. Coloured symbols for a given trait indicate significant differences between F_{ST} and Q_{ST} distributions from Kruskal–Wallis tests, while shaded areas show 95% CIs for F_{ST}.

their ranges (Escudero et al., 2015; Matesanz, Ramos-Muñoz, Blanco-Sánchez, et al., 2020b; Rajakaruna, 2018). Although *L. subulatum* is a gypsum specialist, and as such, variation in gypsum content and soil conditions may be expected to impose strong selection pressures, our results support the interpretation that climatic heterogeneity, rather than soil composition, has driven phenotypic differentiation among populations across the species' range. Specifically, intraspecific variation was not associated either with the content of gypsum or nutrients in the soil, suggesting that soil chemical composition has not been a main driver of phenotypic evolution in *L. subulatum*. In contrast with previous hypotheses that predict strong phenotypic convergence (i.e. a stress resistance syndrome sensu Rajakaruna, 2018), we found substantial phenotypic variation and phenotypic plasticity, indicating that natural selection has not favoured a uniform optimum phenotype across populations to cope with restrictive soil chemical composition.

Previous studies suggested that evolution in isolated and distinctive habitats (such as those with unusual substrates) may be expected to result in reduced levels of within-population genetic variation, limiting microevolutionary potential in edaphic specialists and leading to evolutionary dead-ends (Anacker et al., 2011; Rajakaruna, 2018). In contrast to this expectation, we found footprints of population divergent evolution and substantial quantitative genetic variation both among and within populations, indicating that evolution in gypsum habitats did not eliminate genetic variation in *L. subulatum*. Although climatic differences across populations during seed formation could have caused phenotypic differences associated with differential maternal environmental effects, these effects were minimized in our study by phenotyping plants during their second growth season and including maternal seed mass in the statistical analyses, suggesting that the observed phenotypic differences are genetically based. Accordingly, previous studies have reported significant additive genetic variation for drought-response traits in other gypsophile species (Blanco-Sánchez et al., 2023). Taken together, these results indicate a high potential to respond to climate-driven selection of Iberian gypsophiles, which is likely to have implications for adaptation in the face of rapid ongoing climate change.

Several pieces of evidence support the conclusion that adaptive intraspecific differentiation among populations in *L. subulatum* was likely driven by adaptation to contrasting climatic conditions. First, both univariate and multivariate trait-climate associations showed that quantitative genetic differentiation in *L. subulatum* was strongly related to climatic differences among populations (Figures 2 and 4), even when accounting for the potential effects of the neutral genetic and spatial structure of populations. Second, $F_{ST}-Q_{ST}$ comparisons showed that population differentiation in neutral markers was generally lower than phenotypic differentiation, suggesting that populations of this species have undergone divergent selection. While a larger number of molecular markers would offer a more precise estimate of neutral genetic differentiation, the 10 microsatellite markers we used still serve as a reasonable baseline of neutral variation against which to compare a similar number of phenotypic traits. These results substantiate the role of climatic conditions across the species' range, particularly annual temperature and precipitation, as primary drivers of adaptive differentiation in gypsum specialists.

Our results also provide more information on how variation in climatic conditions have shaped phenotypic differentiation among populations. Specifically, individuals of populations from sites with harsher (i.e. warmer and drier) climatic conditions showed earlier reproductive phenology, which is consistent with an adaptive evolutionary response to escape from drought in Mediterranean-type and semiarid ecosystems, agreeing with previous studies reporting phenological differences among populations associated with climatic differences (Brouillette et al., 2014; Franks et al., 2007; Matesanz, Ramos-Muñoz, Moncalvillo, et al., 2020a). Furthermore, in contrast to expectations for a drought-escape strategy, individuals from populations in drier sites showed significantly higher water use efficiency (WUE; estimated from δ^{13}C) than those from populations in mesic sites under drought conditions (Figure 2a). In Mediterranean species, higher WUE in drier populations due to divergent selection has been reported as an adaptation to minimize water loss (Lázaro-Nogal et al., 2016; Matesanz & Valladares, 2014; Ramírez-Valiente et al., 2010). Our results contrast with the predicted trade-off between high WUE and fast reproductive phenology (Kooyers, 2015). However, such a trade-off does not appear to be universal, as several recent studies have shown that populations can express both early phenologies and high WUE (Brouillette et al., 2014; Kooyers, 2015; Kooyers et al., 2015; and references). Although the processes underlying advanced phenologies and high WUE are far from resolved, previous studies argued that a lack of genetic constraints between traits affecting WUE may result in higher photosynthetic efficiency, consequently allowing early reproductive phenology and a water-saving behaviour (Brouillette et al., 2014; Kooyers, 2015; Kooyers et al., 2015).

Individuals from populations with harsher climatic conditions also showed larger but more sclerophyllous leaves (i.e. greater LA but lower SLA) with lower leaf N content than those from climatically milder populations (Figures 2 and 4). According to predictions from the leaf economics spectrum framework, a positive correlation between LA, SLA, and leaf N is often found, and low values of these traits are expected in harsh environments (e.g. Reich, 2014; Wright et al., 2004). Thus, our results again showed a partial contrast with the pattern predicted by the leaf economics spectrum and with empirical evidence reported in previous studies (Blumenthal et al., 2020; Kooyers et al., 2015). Nevertheless, a similar trait syndrome, with larger and more sclerophyllous leaves with lower leaf N, has been found in populations from drier sites in other Mediterranean species (Ramírez-Valiente et al., 2011, 2014). Previous studies have shown the adaptive value of more sclerophyllous leaves in populations from stressful environments (e.g. Ramírez-Valiente et al., 2011, 2014), because sclerophyllous leaves tend to have smaller cells, thicker walls, and other anatomical features that minimize water loss and photodamage (Blumenthal et al., 2020; Solé-Medina et al., 2022). Although large LA may be associated with high evapotranspiration, several studies have shown that larger leaves may be adaptive for Mediterranean species even under dry conditions (Donovan et al., 2007; Ramírez-Valiente et al., 2011). Larger LA is sometimes associated with greater WUE (e.g. Dudley, 1996), suggesting that natural selection has favoured photosynthetically efficient individuals with an optimum balance between carbon uptake and water loss, in populations with more arid conditions. The adaptive role of lower leaf N under drought has been discussed in terms of photosynthetic efficiency, since leaves with low SLA often lack an effective photosynthetic use of high leaf N because of CO_2 diffusion limitations (Ramírez-Valiente et al., 2014; Reich, 2014).

Previous studies have reported that individuals from populations of drier and warmer areas usually show conservative resource-use strategies to cope with drought (Ramírez-Valiente et al., 2009; Solé-Medina et al., 2022). However, our results agree with other studies that found that intraspecific adaptive differentiation is not easily linked to a particular resource-use strategy. Our study clearly supports the notion that drought-avoidance and drought-escape are not inherently mutually exclusive (Brouillette et al., 2014; Kooyers et al., 2015; Ramírez-Valiente et al., 2011, 2018; Welles & Funk, 2021). Since natural selection acts on multivariate phenotypes, different selective pressures shaping the adaptive responses of populations may favour contrasting resource-use strategies simultaneously, causing discrepancies between the predicted and the observed strategies at the intraspecific level (Anderegg et al., 2021; Kooyers et al., 2015; Solé-Medina et al., 2022). The fact that individuals from populations with hotter and drier climatic conditions expressed both conservative (e.g. higher WUE, lower SLA and leaf N content) and acquisitive trait syndromes (e.g. higher LA and RGR, advanced phenology, and higher plasticity of δ^{13}C and leaf N) suggests that rather than a specific strategy, natural selection favoured particular trait values to cope with drought in *L. subulatum*. These results indicate that the evolution of phenotypes related to contrasting resource-use strategies (acquisitive or conservative) was not genetically constrained in *L. subulatum*, agreeing with previous results from other Mediterranean species (Kooyers et al., 2015; Ramírez-Valiente et al., 2011) and with studies that have discussed the relatively minor role of genetic constraints compared with selection in the evolution of the phenotype (Donovan et al., 2011).

Individuals from populations with harsher climatic conditions showed consistently higher reproductive biomass in both watering treatments, indicating that adaptation to harsh environmental conditions was not coupled with a fitness trade-off across conditions (Hereford, 2009; Matesanz, Ramos-Muñoz, Blanco-Sánchez, et al., 2020b). In contrast, individuals from populations with milder climatic conditions showed low reproductive fitness, especially under drought conditions, suggesting that these populations may be vulnerable to climatic changes.

Patterns of phenotypic plasticity and multivariate analyses showed that plastic responses to drought were generally similar across populations, suggesting that natural selection may have favoured similar plasticity across populations (i.e. homogenizing selection on plasticity; Pigliucci & Kolodynska, 2002). However, plasticity patterns for some traits varied among populations. Specifically, populations from warmer and drier sites expressed high plasticity for two key drought-response traits, WUE and N (Figure 3). It has been proposed that "water-wise" populations save water only under drought conditions, maximizing water use when it is abundant (Nicotra & Davidson, 2010). Therefore, the higher WUE and leaf N plasticity of climatically harsher populations may also reflect a more efficient photosynthetic and resource-use capacity, contributing to the higher reproductive fitness observed. We found both similar and contrasting plastic responses among populations, suggesting that selection on plasticity may be both trait- and environment-dependent. Further research is needed to pinpoint the processes that underlie the striking patterns of plasticity in *L. subulatum*. Nevertheless, the fact that all populations expressed highly plastic responses suggests that selection has not acted against plasticity. In contrast to prior hypotheses and some previous results (Solé-Medina et al., 2022; Valladares et al., 2007), our results highlight the fact that stressful environments do not necessarily benefit more canalized phenotypes. High plasticity across populations may have had relevant evolutionary consequences for gypsophiles. It likely allowed these species to cope with the stressful and variable environments of gypsum habitats while maintaining high within-population genetic variation. In turn, this high variation could have facilitated adaptive evolution in response to climatic heterogeneity (Gomez-Mestre & Jovani, 2013; Matesanz, Ramos-Muñoz, Blanco-Sánchez, et al., 2020b).

Overall, our study demonstrates that the unique substrate conditions of gypsum soils have not constrained the ability of *L. subulatum* to adapt to climatic differences among populations, emphasizing the importance of climate, rather than soil features, as a key driver of adaptive intraspecific variation. Individuals from populations in drier and warmer sites were adapted to more arid climatic conditions and showed higher fitness in both watering treatments, suggesting that populations from colder and more mesic sites will likely be more vulnerable in the context of climate change. Our findings have evolutionary and ecological implications for the expected responses of gypsophiles to the ongoing environmental changes in the Mediterranean region, since they highlight that the ability to adapt to future climatic conditions is not necessarily limited in substrate endemic species.

AUTHOR CONTRIBUTIONS

Silvia Matesanz conceived the idea; Mario Blanco-Sánchez, Marina Ramos-Muñoz, Beatriz Pías, and Silvia Matesanz collected the data; Mario Blanco Sánchez, José Alberto Ramírez-Valiente, and Steven J. Franks analysed the data; all authors discussed the main results; Mario Blanco-Sánchez led the writing of the manuscript. All authors critically contributed to the drafts and gave final approval for manuscript publication.

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CONFLICT OF INTEREST STATEMENT

None declared.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14322.

DATA AVAILABILITY STATEMENT

Phenotypic and genetic data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.f1vhhmh4c (Blanco-Sánchez et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Mean values of environmental variables (climate and soil composition) of the 11 sampled populations of *Lepidium subulatum* L. and the CULTIVE facilities (URJC), where the common garden experiment was established.

Table S2. Results from the Pearson's pairwise correlations between the main environmental variables and the geographical location (latitude and longitude) of populations.

Table S3. Results from the Mantel correlogram to test for significant associations between geographical and environmental distances between populations.

Table S4. Results from linear mixed models testing the effect of population (df = 10), treatment (i.e. phenotypic plasticity; df = 1) and their interaction (P × T; df = 10) in the phenotypic expression.

Table S5. Results from the Mantel correlogram to test for significant associations between geographical and genetic distances (pairwise F_{ST}), that is, Isolation by Distance (IBD), and from the Mantel test to evaluate the association between environmental and genetic distances between populations, that is, Isolation by Environment (IBE).

Table S6. Results from partial Mantel tests to assess significant associations between phenotypic and environmental distances between populations, while controlling for genetic and geographic distances (i.e. neutral processes).

Methods S1. (a) Detailed description of rain exclusion structures and mean environmental conditions registered below and outside the structures. (b) Experimental individuals of *Lepidium subulatum* growing below the rain exclusion structures.

Figure S1. Soil water content (%) in each of the three rain exclusion structures for both watering treatments (well-watered and drought; WW and DRO, respectively).

Figure S2. Phenotypic variation of populations across watering treatments.

Figure S3. Results from univariate associations between quantitative genetic differentiation among populations in each treatment and the environmental conditions of populations (climate and soil composition).

Figure S4. Principal component analysis (PC1 vs. PC3) used to summarize environmental variables (climate and soil composition) of populations.

Figure S5. Results from multivariate associations between the two first axes of the phenotypic PCA performed to summarize quantitative genetic differentiation among populations in both treatments (well-watered and drought; WW and DRO, respectively), the magnitude and direction of multivariate plasticity, and the environmental PCA used to summarize climatic and soil composition differences among populations.

Figure S6. Results from the PCA used to calculate multivariate phenotypic plasticity.

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