Drought and parental effects shape plant responses to decreased precipitation predictability

Martí March-Salas[1,2,3*], Lucrezia Laccetti[1*], Linda Eberhardt[3,4], Charlotte Mundt[3], Vera Holland[3], Patrick S. Fitze[5,6], J.F. Scheepens[3]

[1] *Area of Biodiversity and Conservation, Department of Biology, Universidad Rey Juan Carlos, ESCET, Móstoles, Madrid, Spain.*

[2] *Instituto de Investigación en Cambio Global (IICG-URJC), Universidad Rey Juan Carlos, Móstoles, Madrid, Spain.*

[3] *Plant Evolutionary Ecology, Faculty of Biological Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany.*

[4] *Department of Molecular Ecology, Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany.*

[5] *Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, Spain.*

[6] *Department of Biodiversity and Ecologic Restoration, Instituto Pirenaico de Ecología (IPE-CSIC), Jaca, Spain.*

* Corresponding authors: Martí March-Salas (marti.march.salas@urjc.es) and Lucrezia Laccetti (lucrezia.laccetti@urjc.es).

This manuscript is a preprint and has not been peer reviewed.

Abstract

Current climate change is leading to an increase in the frequency and intensity of droughts and to decreased precipitation predictability, both potentially affecting the persistence of plant populations. Precipitation predictability has been shown to shape plant phenotypic variation and drive the evolution of phenotypic plasticity, yet whether differences in precipitation predictability also trigger parental environmental effects, and whether drought modifies their expression, remains unknown. To fill this gap, we performed a controlled greenhouse experiment on offspring of *Papaver rhoeas* L. (Papaveraceae) whose maternal lines had been exposed to more *versus* less predictable precipitation during three consecutive generations in an outdoor field experiment. Offspring were exposed to more or to less predictable precipitation, crossed with a control and a drought treatment, which allowed us to test whether offspring perform better under parental than under non-parental predictability regimes, and whether drought intensifies these responses. We measured growth-related (growth rate, total biomass), morphological (leaf length, specific root length) and physiological (chlorophyll content, photosynthetic performance) traits of the offspring. We found that contrasting levels of offspring precipitation predictability led to significant differences in growth rate, total biomass, leaf length and specific root length, whereas drought reduced plant growth independently of offspring predictability and modulated predictability effects on chlorophyll content. Offspring plants from the lower parental predictability treatment grew faster under less predictable compared to more predictable precipitation, indicating parental environmental effects on offspring growth responses. Overall, our study highlights that precipitation predictability is an important driver shaping plant phenotypic responses both within and across generations, and that its transgenerational effects are expressed mainly when both parents and offspring experience less predictable precipitation, while drought, which is

expected to become increasingly frequent under climate change, can modulate these responses.

Keywords: Drought stress, indoor *versus* outdoor experiments, intrinsic precipitation predictability, parental environmental effects, phenotypic plasticity, transgenerational plasticity.

Introduction

Phenotypic variation is key for the evolution of plant populations, and phenotypic differences among individuals of the same species can arise from underlying genetic variation or from plastic responses to the environment (Mazer and Gorchov, 1996; Uller et al., 2013; Wu et al., 2026). Plant phenotypes can also be influenced by the environmental conditions experienced by their parents, a phenomenon referred to as parental environmental effects or transgenerational phenotypic plasticity (Herman and Sultan, 2011; Burgess and Marshall, 2014; Latzel et al., 2023). Such parental environmental effects can help offspring to withstand stressful environmental conditions, but they can also lead to weak or even negative effects on offspring fitness (e.g., Uller et al., 2013; Baker et al., 2019; Latzel et al., 2023). Although parental environmental effects have been extensively examined regarding drought, elevated temperature, light intensity, nutrient availability, as well as infections caused by pathogens or microbes (e.g., Galloway and Etterson, 2007; Groot et al., 2017; Puy et al., 2021; Latzel et al., 2023), the effects of environmental predictability remain largely underinvestigated.

Current climate change leads, among other effects, to increased drought, greater environmental variability, and reduced environmental predictability (IPCC, 2014). Reduced environmental predictability increases uncertainty in the amount and timing of resources, which may alter the phenotypic response of plant populations. Theoretical studies have shown that environmental predictability is a crucial factor influencing parental effects and plant evolutionary processes (Reed et al., 2010; Proulx and Teotónio, 2017). In recent years, studies have shown that transgenerational responses to environmental predictability can occur rapidly (Franch-Gras et al., 2017; March-Salas et al., 2019, 2022; Leung et al., 2020; Yin et al., 2022). Under less predictable conditions simulated over several years, descendant plants exhibited rapid phenotypic changes (from one generation to the next) leading to increased

fitness, whereas plants in more predictable environments required multiple generations to display a similar response (March-Salas et al., 2019). Rapid transgenerational plastic responses have been demonstrated with respect to environmental predictability (March-Salas et al., 2019, 2021b) and also with respect to other environmental factors (e.g., nutrient addition; Yin et al., 2022). However, whether offspring plastic responses differ when offspring experience environmental conditions that match or mismatch those experienced by parental plants has rarely been investigated. Yet, explicitly exposing offspring to parental and non-parental environments is crucial to assess whether parental effects are adaptive, which is still debated (Badyaev, 2008; Herman and Sultan, 2011; Burgess and Marshall, 2014; Latzel et al., 2023).

While environmental factors that significantly affect the parents can determine parental environmental effects in their offspring, factors that have little or no effect on the parental generation are generally assumed not to trigger a response in their offspring (Uller et al., 2013; Latzel et al., 2023). However, non-significant trends observed in the parental generation can be magnified in the subsequent generation if parents and offspring are exposed to similar conditions, potentially resulting in parental environmental effects (Ren et al., 2022). Parents may thus pre-adapt offspring to the same environmental conditions and consequently enhance the phenotypic trait expression of their offspring, thereby leading to significantly different offspring responses (Baker et al., 2019). Such anticipatory parental effects are more likely expressed during early stages of plant development (Burgess and Marshall, 2014), since maternal effects (*i.e.*, parental environmental effects transmitted through the mother, such as maternal provisioning) usually fade away with plant growth (Roach and Wulff, 1987; Latzel et al., 2010). This cross-generational amplification may be particularly relevant for environmental predictability, which, unlike acute stressors such as drought, flooding, or heat

100 waves, may exert relatively subtle effects within a single generation. Phenotypic
101 differentiation in response to contrasting levels of predictability may therefore become more
102 pronounced after exposure over successive generations (March-Salas et al., 2019).

103 Most environmental changes manifest as a combination of several environmental
104 factors (e.g., changes in precipitation predictability can result in drought, and more
105 precipitation generally leads to cooler air-temperatures) and experiments manipulating several
106 rather than a single environmental factor are needed to understand the relevance of parental
107 effects. A recent large experiment simultaneously manipulated 24 different biotic and abiotic
108 factors to study parental environmental effects (Latzel et al., 2023) and demonstrated the
109 relevance of manipulating several factors at the same time. However, environmental
110 predictability was manipulated neither in this study nor in other studies investigating parental
111 effects across multiple environments (e.g., Bossdorf et al., 2009; Latzel et al., 2010;
112 Münzbergová et al., 2017; Lampei, 2019). Lower parental precipitation predictability may
113 allow plants to withstand droughts, because it typically entails less regular water availability,
114 which may pre-adapt plants to fluctuating water availability (Springer et al., 2024). Moreover,
115 drought could exacerbate parental environmental effects caused by differences in precipitation
116 predictability, because drought leads to additional stress, and therefore exposure to drought
117 may hinder compensatory strategies.

118 In this study, we experimentally tested whether different past precipitation predictability
119 regimes caused different parental environmental effects and whether parents and offspring
120 exposed to the same or different predictability regimes caused different phenotypic offspring
121 responses. Moreover, offspring were simultaneously exposed to drought or control conditions
122 to test whether additional stress magnified parental environmental effects. To this end, we
123 used maternal lines of the widespread annual herb *Papaver rhoeas* L. (Papaveraceae) that

were experimentally exposed in the field to more or to less predictable precipitation regimes over three consecutive generations. Seeds of both types of maternal lines were sown in a controlled greenhouse experiment to study whether parental precipitation predictability promotes different phenotypic offspring responses. To investigate whether offspring thrive better in parental than in non-parental environments, offspring from each maternal line were exposed to both more and less predictable precipitation regimes. Half of the offspring in each parental × offspring predictability combination were additionally exposed to drought, to investigate whether drought intensifies the effects induced by parental and offspring precipitation predictability. In the offspring, we measured growth-related (growth rate and total biomass), morphological (leaf length and specific root length) and physiological traits (chlorophyll content and photosynthetic performance). Specifically, we hypothesized: (i) that the degree of precipitation predictability experienced by parents drives the phenotypic response of offspring plants; (ii) that offspring exposed to less predictable precipitation show enhanced performance (e.g., faster growth and higher biomass) when the parental environment was also less predictable, whereas a more predictable parental environment does not result in divergent responses in their offspring; (iii) that precipitation predictability effects in the offspring generation are congruent with those in the parental generation; and (iv) that drought intensifies the effects of parental and offspring precipitation predictability.

Materials and methods

Study species

Papaver rhoeas L. (common poppy, Papaveraceae) is a cosmopolitan and fast-growing annual herb. It has a widespread distribution, inhabiting Europe, central and northern Asia, and North

146 Africa (McNaughton and Harper, 1964), where it mainly grows in arable fields, particularly
147 cereal fields, and in other disturbed habitats such as field margins and roadsides (McNaughton
148 and Harper, 1964; Torra et al., 2011). This species exhibits a fast resource-acquisitive strategy
149 and high phenotypic plasticity (McNaughton and Harper, 1964; Pérez-Ramos et al., 2019). It
150 has a waxy cuticle and hairy leaves that help the plants to reduce water loss through
151 transpiration. The basal leaves form a rosette arrangement, and alternate leaves develop along
152 the stem (Tutin et al., 1993). It also possesses a taproot system with several secondary root
153 branches (McNaughton and Harper, 1964). All these features allow *P. rhoeas* to efficiently
154 thrive in dry environments (Torra et al., 2011; Pérez-Ramos et al., 2019).

155 ***Precipitation predictability treatments in parental plants***

156 In 2012, *P. rhoeas* seeds were sown at the outdoor experimental station 'El Boalar' (42°33'N,
157 0°37'W, 705 m.a.s.l.; Jaca, Spain), and the plants and their descendants were exposed over
158 three consecutive generations (2012–2014; see below) to different levels of intrinsic
159 precipitation predictability through treatment-specific patterns of supplemental precipitation
160 delivered by an automated watering system (March-Salas et al., 2019; March-Salas and Fitze,
161 2019a). Plants were exposed either to more (M_p) or to less (L_p) predictable precipitation (the
162 subscript 'p' indicates that the treatment was applied to the parental lineages in the field, as
163 opposed to the offspring treatment applied in the greenhouse experiment; see Experimental
164 setup), by means of regular (M_p treatment) and random time-intervals (L_p treatment) of
165 supplemental watering events, in addition to the natural precipitation. This sprinkling regime
166 is hereafter referred to as the 'parental predictability' (PP) treatment. Both treatments
167 consisted of the same total amount of precipitation (natural + supplemental precipitation) and
168 of 14 supplemental watering events per week (each of 1.3 mm), and the same watering

169 treatment was applied to each generation from April to the end of September. As a result, total
170 precipitation exhibited a weighted permutation entropy (*i.e.*, a measure inversely related to
171 intrinsic predictability; Pennekamp et al., 2019) of 0.77 in M_p and 0.86 in L_p. Intrinsic
172 precipitation predictability in M_p was equal to the one of the natural precipitation, and the
173 intrinsic precipitation predictability of L_p was 11.69% lower than the one of M_p and the
174 natural precipitation (March-Salas et al., 2019). Each treatment was applied to four plots of
175 7.2 m^2, with each plot established in a separate 100 m^2 enclosure containing natural
176 vegetation. Supplemental precipitation was delivered by four sprinklers, one in each corner of
177 the enclosure (*see* Fig. S1). Plots were surrounded by an exclusion net (30 cm aboveground
178 and 10 cm belowground) that protected the plants from herbivory by snails and slugs, and
179 enclosures were separated with a 1 m tall metal wall and a mesh, preventing predation and
180 limiting outcrossing.

181 To test whether precipitation predictability treatments caused transgenerational
182 phenotypic changes, these treatments were applied over three consecutive generations in such
183 a way that offspring seeds were always sown under the same precipitation predictability
184 treatment, but never in the plot of the maternal plant, to avoid the potential confounding effect
185 of plot-specific conditions. The original seed lot, collected in 2011 from 50 maternal plants of
186 a natural population located approximately 60 km from the experimental site and
187 experiencing similar climatic conditions, was sown in 2012 (G0). Seeds generated in 2012
188 (G1) were sown in 2013 and raised under the same treatment as their ancestors (G0), and
189 seeds generated in 2013 (G2) were sown in 2014 and raised under the same treatment as their
190 ancestors (G0 and G1; March-Salas et al., 2022; Springer et al., 2024). Finally, the seeds
191 generated in 2014 (G3) were collected, separated by maternal plant and stored in dry and dark
192 conditions. Seed collection and storage were standardized and conducted by the same person

193 across years, and all seeds were collected at the same stage of capsule development (March-
194 Salas et al., 2022). Since *P. rhoeas* is self-incompatible (Lawrence, 1975), the collected seeds
195 resulted from cross-pollination. Seeds were collected from several flowers of the same
196 maternal plant, and these flowers may have been pollinated by different pollen donors, so that
197 the seedlings produced could be either half- or full-siblings. Because plots of the two PP
198 treatments were located in separate enclosures, the spatial separation limited outcrossing
199 between treatments, so that maternal plants and pollen donors most likely experienced the
200 same PP treatment. Hence, the term 'parental' is used throughout to refer to the precipitation
201 predictability treatment experienced by the parental generation.

202 To compare the phenotypic responses of the parental and offspring generations, we
203 used data collected in the field on the maternal plants of the offspring studied here (*i.e.*, the
204 G2 plants grown in the field plots in 2014). In these maternal plants, the maximum rosette
205 diameter (mm) was measured weekly from germination until the end of the plant life cycle,
206 and 'rosette diameter growth rate' (hereafter 'growth rate') was calculated by subtracting the
207 initial from the final diameter and dividing the difference by the number of weeks elapsed
208 between both measurements. For each maternal plant separately, seeds were collected,
209 counted and stored in dry and dark conditions. After seed collection, the entire plant was
210 collected and separated into roots and above-ground parts. The maximum root length was
211 measured to the nearest 1 mm and the aboveground and belowground dry biomasses were
212 weighed after drying in an oven for 72 hours at 60°C. Total dry biomass (hereafter 'total
213 biomass') refers to the sum of the root and the aboveground dry biomasses. Finally, Specific
214 Root Length (SRL) was calculated (maximum root length / dry belowground biomass).

215 ***Experimental setup***

216 In winter 2021–2022, an experiment with the offspring seeds (G3 seeds generated by G2
217 maternal plants grown in the field plots) was conducted under controlled conditions in a
218 greenhouse at Goethe University Frankfurt (50°10'N, 8°38'E, 123 m.a.s.l.; Frankfurt am
219 Main, Germany). Five seed families from each PP treatment were randomly selected,
220 resulting in ten seed families in total, which originated from five of the eight plots (two L_p and
221 three M_p plots; one to three seed families per plot). Seeds were sown in December 2021 in
222 separate trays (one tray per seed family) with standard potting soil (Type T1b, Hawita
223 Fruhstorfer Erde), and irrigated with sufficient water. In January 2022, twelve replicates per
224 seed family were randomly selected and transplanted into 0.75 L pots, resulting in a total
225 number of 120 plants, *i.e.*, twelve plants per seed family. On the transplanting day, the largest
226 distance between the tips of two leaves (in mm), hereafter referred to as "maximum rosette
227 diameter (mm)", was measured in order to account for potential effects of the initial size. The
228 pots were randomly placed on a table in the greenhouse and the position of the pots was
229 randomized once per week. In the next four weeks, half of the seedlings of each seed family
230 were exposed to more (M_o; the subscript 'o' indicates that the precipitation predictability
231 applies to the offspring) and the other half to less (L_o) predictable conditions (hereafter
232 referred to as 'offspring predictability' treatment; OP). Plants from both OP treatments
233 received the same total amount of water, delivered in three watering events per week. In M_o,
234 the three watering events were applied once per day on Monday, Wednesday and Friday,
235 while in L_o, the three watering events were randomly applied along the week, so that zero to
236 three watering events could occur on a single day. Additionally, a drought treatment was
237 applied to half of the pots of each predictability treatment group (*i.e.*, of each PP × OP
238 combination), and thus to half of the seedlings of each seed family. Pots of the control

239 treatment received the full amount of water described above (hereafter 'control conditions'),
240 whereas pots of the drought treatment received 50% less water (hereafter 'drought'). Each
241 seed family was therefore represented by six plants per OP treatment and by three plants per
242 combination of OP and drought treatment. During the entire greenhouse experiment, the
243 temperature (°C) and illuminance (lx) were recorded every 15 minutes with three data loggers
244 (HOBO UA-002-08 Pendant Temp/Light, Onset®, USA) randomly placed among the pots. A
245 photoperiod of 12 hours of light per day was maintained, and the average temperature was
246 24°C during the day and 16°C at night.

247 ***Offspring phenotyping***

248 At the end of the greenhouse experiment, we measured the maximum rosette diameter (in
249 mm; used to calculate growth rate) and the length of the longest leaf (in mm; hereafter 'leaf
250 length'). Growth rate was calculated as described above for the maternal plants, but dividing
251 by the number of days elapsed between both measurements (i.e., in mm/day). The average
252 relative leaf chlorophyll content (SPAD units; hereafter referred to as 'chlorophyll content')
253 was measured in three leaves using the Chlorophyll Meter SPAD-502 Plus (Konica-Minolta,
254 Japan). Chlorophyll *a* fluorescence of Photosystem II was measured at night with a portable
255 chlorophyll fluorimeter (Pocket-PEA, Hansatech Instruments Ltd., UK), and we then used the
256 PEA Plus 1.0.0.1 software with 50 µs of fluorescence level as F_0 (the ground fluorescence) to
257 calculate the Performance Index (PI_{abs}). The Performance Index is a mathematical term
258 combining the effects of light trapping, the maximum quantum efficiency of the Photosystem
259 II and the probability that an absorbed photon leads to reduction further than Q_A (primary
260 acceptor plastoquinone; Strasser et al., 2000, 2004) and refers to the functionality of the plant
261 photosynthetic apparatus. The Performance Index is widely used as an indicator of plant

vitality because of its sensitive response to abiotic stress in plants (e.g., Jedmowski et al., 2013; Bantis et al., 2020), so we hereafter refer to this measure as 'photosynthetic performance'. The plants were then harvested and separated into roots and above-ground parts. The substrate adhered to the roots was removed carefully, and the length of the longest root was measured to the nearest 1 mm as an estimate of maximum rooting depth. Both dry aboveground and belowground biomass (mg) were determined, and total biomass and SRL were calculated as described above.

Statistical analysis

All statistical analyses were conducted with R *version* 4.0.3 (R Core Team, 2020). First, we tested whether offspring phenotypes differed between PP, OP, and drought treatments. To this aim, Linear Mixed-effect Models (LMM) were applied for each of the six plant traits (growth rate, total biomass, leaf length, SRL, chlorophyll content and photosynthetic performance), using the 'lme4' package and the 'lmer' function (Bates et al., 2015). Parental predictability (PP; M_p *versus* L_p), offspring predictability (OP; M_o *versus* L_o) and drought treatment (D; control *versus* drought) were included as fixed factors, as well as their two- and three-way interactions. Seed family (nested in plot of origin for growth rate, total biomass and photosynthetic performance) was included as random factor and initial rosette diameter as covariate. The significance of the fixed terms was assessed by means of type II Wald chi-square tests, using the 'Anova' function of the 'car' package (Fox and Weisberg, 2019). Second, we tested for effects of precipitation predictability on the maternal phenotypes (*i.e.*, on the plants growing in the field experiment in 2014). To this end, we conducted three separate LMMs, one for growth rate, one for total biomass and one for SRL of the maternal plants. The LMMs included PP (M_p *versus* L_p) as fixed factor and plot as random factor. We

285 used models with a Gaussian error distribution, so we tested the assumptions of normality and
286 homogeneity of variance of the residuals using the Shapiro-Wilk test and the Bartlett test,
287 respectively. If these assumptions were not met, we transformed the response variable (*see*
288 transformations in Table 1). We applied Tukey's post-hoc contrasts on the model estimates
289 for two- and three-way interactions using the 'lsmeans' package (Lenth, 2016), and estimated
290 effect sizes as differences between estimated marginal means on the original scale, with 95%
291 confidence intervals (Table S1). Post-hoc contrasts are reported for all traits to allow
292 comparisons among treatment combinations, but they were interpreted with caution where the
293 corresponding interaction was not significant. We decomposed the three-way interaction on
294 chlorophyll content by testing the OP × D interaction within each PP treatment (Table S1).
295 The graphs were created using the 'ggplot2' package (Wickham, 2016).

296 **Results**

297 ***Effects of parental predictability treatment on offspring phenotypes***

298 Parental predictability alone did not significantly affect any offspring trait, but it modulated
299 the offspring response to OP in two of the six traits: the PP × OP interaction was significant
300 for growth rate, and the PP × OP × D interaction for chlorophyll content (Table 1). Moreover,
301 post-hoc contrasts showed that OP treatments differed in offspring from L_p for three traits
302 (growth rate, total biomass and leaf length) and marginally for SRL, but never in offspring
303 from M_p (Fig. 1). Growth rate depended on the PP × OP interaction (P = 0.038), with
304 offspring from L_p growing significantly faster in L_o than in M_o (post-hoc contrast: P = 0.024;
305 Fig. 1A). Total biomass was greater in L_o than in M_o (OP effect: P = 0.003), and in post-hoc
306 contrasts this difference was significant only in offspring from L_p (P = 0.022; Fig. 1B). Leaf

length was greater in L_o than in M_o (OP effect: P = 0.005) and tended to be greater in L_p than in M_p offspring (PP effect: P = 0.070). This tendency stemmed from L_p–L_o offspring, which had significantly longer leaves than L_p–M_o (post-hoc contrast: P = 0.014) and M_p–M_o offspring (P = 0.017), whereas OP treatments did not differ in offspring from M_p (Fig. 1C). SRL was significantly lower in L_o than in M_o (OP effect: P = 0.007), and this difference tended to be significant only in offspring from L_p (post-hoc contrast: P = 0.051; Fig. 1D). Chlorophyll content depended on the three-way PP × OP × D interaction (P = 0.006), so its main effects and two-way interactions are not interpreted (Fig. 1E; see below and Fig. S2). Photosynthetic performance tended to be higher in offspring from L_p (PP effect: P = 0.062), but no post-hoc contrast was significant (Fig. 1F). Effect sizes for all traits are given in Table S1.

Effects of precipitation predictability on parental versus offspring generation

In the outdoor experiment conducted on the parental generation in 2014, there were no significant differences in growth rate (χ^2 = 0.026; P = 0.871), total biomass (χ^2 = 1.607; P = 0.205) and SRL (χ^2 = 0.057; P = 0.811) between M_p and L_p (Fig. 2A–C). In contrast, in the greenhouse experiment conducted on the offspring generation in 2022, growth rate and total biomass were significantly higher and SRL was significantly lower in L_o than in M_o (main effect of OP; Table 1; Fig. 2D–F); for growth rate, however, this difference was only significant in offspring from L_p (PP × OP interaction; Fig. 1A). In addition, OP significantly affected leaf length, which also tended to differ between PP treatments, chlorophyll content was affected by the PP × OP × D interaction, and photosynthetic performance was not significantly affected by any treatment (Table 1).

Influence of drought on the plant response to precipitation predictability

Drought significantly reduced growth rate ($P = 0.002$), total biomass and leaf length, and increased SRL (all $P < 0.001$), independently of OP and PP (OP × D and PP × D: all $P \geq 0.2$; Table 1; Fig. 3A–D), while it did not affect photosynthetic performance ($P = 0.847$; Fig. 3F). Although the OP × D interaction was not significant, post-hoc contrasts showed that total biomass and SRL tended to differ between L_o and M_o under drought ($P = 0.064$ and $P = 0.057$, respectively), but not under control conditions (Fig. 3B, D). Chlorophyll content was the only trait for which the effect of drought depended on the predictability treatments (three-way PP × OP × D interaction; Table 1; Fig. S2). This interaction arose because the effect of drought depended on OP in offspring from L_p (OP × D interaction within L_p: $P = 0.011$) but not in offspring from M_p ($P = 0.184$): in offspring from L_p, chlorophyll content tended to be higher in M_o than in L_o under control conditions but lower under drought, whereas offspring from M_p showed the opposite, non-significant pattern. Drought increased chlorophyll content only in L_p–L_o offspring (post-hoc contrast: $P = 0.012$), whereas drought and control conditions did not differ in the other three PP × OP combinations (Fig. S2).

Discussion

Parental environmental effects are widely recognized to be triggered by multiple environmental factors (e.g., Latzel et al., 2023). However, it is largely unknown whether environmental predictability shapes parental effects, and whether such effects could confer an advantage to plant populations facing increasingly unpredictable and dry environmental conditions. Overall, our study shows that precipitation predictability can trigger parental effects in *P. rhoeas*. Specifically, offspring from L_p differed between OP treatments (they

grew faster, had greater total biomass and longer leaves, and tended to have lower SRL in L_o than in M_o), whereas no such differences were found in offspring from M_p. Also, the within-generation effects of precipitation predictability on the measured traits were larger in the offspring generation (OP treatment) than in the parental generation (PP treatment), suggesting that parental effects may amplify phenotypic differences across generations (see below). Finally, drought reduced growth rate, total biomass and leaf length and increased SRL independently of OP and PP, and it modulated the combined effects of PP and OP on chlorophyll content (three-way interaction), indicating that multiple environmental factors jointly affect plastic within- and transgenerational responses (Lampei, 2019).

Does parental precipitation predictability shape the offspring phenotype under controlled indoor conditions?

In line with our first hypothesis, the degree of precipitation predictability experienced by parental plants influenced the phenotypic response of offspring grown in the controlled indoor experiment. Independently of OP, offspring from L_p only tended to have longer leaves and higher photosynthetic performance (marginal main effects of PP; Table 1; Fig. 1C, F). In combination with OP, however, offspring from L_p had a higher growth rate, greater total biomass and longer leaves, and tended to have lower SRL, in L_o than in M_o, whereas no such differences were found in offspring from M_p (Fig. 1A–D); these differences were supported by a significant PP × OP interaction for growth rate and by post-hoc contrasts for the other traits. In addition, drought increased chlorophyll content only in L_p–L_o offspring (Fig. S2). Previous studies showed that plant growth rates and flowering phenology tend to accelerate both in more fluctuating environments (Chapin et al., 1993; Matsubara, 2018) and under lower environmental predictability (March-Salas et al., 2019; Springer et al., 2026). Here, L_p–

374 L_o offspring showed the highest growth rate, total biomass and leaf length and the lowest SRL
375 (Fig. 1A–D). Decreased SRL due to less predictable and irregular precipitation may result
376 from plant investment in lateral roots rather than root length (March-Salas et al., 2021b). Such
377 a shift from primary root elongation towards lateral branching is the strategy expected in
378 annual species such as *P. rhoeas*, which exploit the water available close to the soil surface
379 (March-Salas et al., 2021b, 2022). Lateral roots can promote fast acquisition of water
380 resources in the upper soil layers (von Wangenheim et al., 2020), whereas investing in deeper
381 roots can be an optimal strategy when a more constant water supply recharges deeper soil
382 layers, or when plants prioritize stability and anchorage over immediate performance (Hajek
383 and Knapp, 2022). This result is in line with studies showing that root traits can respond
384 rapidly to fluctuations in water availability and can be under strong selection (Zhou et al.,
385 2019; March-Salas et al., 2021b; Yamauchi et al., 2021).

386 Offspring from L_p tended to have higher photosynthetic performance than offspring
387 from M_p. This tendency may reflect an enhanced functionality of the photosynthetic
388 apparatus, which may have contributed to the faster growth of L_p–L_o offspring and may
389 indicate higher plant vitality (Bantis et al., 2020). A previous study showed that less
390 predictable precipitation alters a plant's physiological performance (March-Salas and Fitze,
391 2019b) and that under less predictable precipitation more nitrogen was incorporated into the
392 leaves, a chemical trait that increases the photosynthetic capacity of plants and individual
393 plant growth and, ultimately, promotes plant persistence to environmental changes (Chapin et
394 al., 1993; Craine et al., 2001; Muñoz-Huerta et al., 2013).

Do offspring perform better under the parental than under a non-parental predictability regime?

Offspring exposed to a predictability regime different from the parental one (L_p–M_o and M_p–L_o, considered together) did not perform consistently worse than offspring exposed to the parental one (L_p–L_o and M_p–M_o, considered together) in any of the six traits (Fig. 1). A strictly anticipatory interpretation, under which a match between the parental and the offspring environment should confer an advantage, is therefore not supported by our data. Instead, exposure of parental plants to less predictable precipitation appears to confer an advantage that is expressed mainly when the offspring also experience less predictable precipitation: offspring from L_p grew better, and tended to have lower SRL, in L_o than in M_o (Fig. 1A–D), whereas no such differences were found in offspring from M_p. This finding is in line with our second hypothesis (*i.e.*, that offspring exposed to less predictable precipitation would show enhanced performance when the parental environment was also less predictable) and with findings on the parental generations published earlier (e.g., March-Salas et al., 2019, 2021a, 2021b, 2022; March-Salas and Fitze, 2019a). It suggests that, in response to a stressful environment, parents confer on their offspring a response that may increase the offspring's fitness, indicating that parental environmental effects induced by lower predictability have the potential to become adaptive (Marshall and Uller, 2007; Herman and Sultan, 2011; Burgess and Marshall, 2014). This is also in line with the longer survival under severe drought reported for plants derived from the same field experiment (Springer et al., 2024). Less predictable precipitation entails a less regular water supply, which may prime plants against subsequent water shortage, so that the offspring inherit a physiological state that anticipates the stress experienced by their progenitors and thereby enhances their performance (Hilker and Schmülling, 2019).

Are the effects of precipitation predictability congruent between the parental and the offspring generation?

Precipitation predictability led to significant phenotypic differences in the offspring generation but not in the parental generation (Fig. 2), even though the direction of the treatment effects was the same in both generations for the three traits that were measured in each of them. This contradicts our third hypothesis, *i.e.*, that exposing parents and offspring to the same precipitation regimes should lead to congruent results. It has been assumed that environmental factors that have no impact on parents should not affect offspring (Latzel et al., 2023). Two explanations may account for why parental exposure to differences in precipitation predictability nevertheless affected the measured offspring traits. First, small non-significant differences in parental means may have been amplified in the following generation, leading to detectable parental effects (Ren et al., 2022).

Second, in the field experiment, the treatment was applied continuously from germination until the completion of the phenological cycle (March-Salas et al., 2021b). In contrast, in the indoor experiment the treatment was applied only for four weeks, ending before flowering. The absence of significant differences in the parents may therefore reflect compensatory mechanisms, since the traits considered were measured at the end of the plants' life, when early treatment differences may already have been fully compensated; a time-resolved analysis of the weekly field measurements would be needed to test this explicitly. The offspring, in contrast, were phenotyped at an early developmental stage, at which parental effects are most likely to be expressed (Latzel et al., 2010; Burgess and Marshall, 2014). However, earlier studies showed that parental *P. rhoeas* plants under less predictable field conditions had higher fitness triggered by earlier flowering, higher survival and heavier seeds (March-Salas et al., 2019; March-Salas and Fitze, 2019a). The lack of effects on the traits

443 measured here suggests that precipitation predictability affected fitness correlates rather than
444 the morphology of the parental plants, and that the parental effects observed in the offspring
445 may have been triggered by differences in these fitness-relevant traits. Overall, these results
446 suggest that the responses to environmental predictability were similar in direction in the
447 outdoor and the indoor experiment.

448 Differences in the magnitude of the predictability effect between the outdoor and
449 indoor experiment may exist due to differences in specific environmental conditions. In their
450 meta-analysis, Poorter et al. (2016) showed that, despite lower day light and temperature,
451 controlled indoor experiments led to stronger effects on growth rates and increased
452 accumulated biomass, probably due to the more benign conditions in indoor experiments.
453 Moreover, many environmental parameters are controlled in indoor experiments, while in the
454 outdoor experiments they cannot be controlled and thus may reduce the magnitude of the
455 treatment effects. This may also explain the different magnitudes of the predictability effects
456 on growth rate and total biomass between the indoor and outdoor experimental system. In
457 addition, soil evapotranspiration was high in the field but lower in the indoor experiment,
458 which may have changed how the timing of watering events translated into soil water
459 availability and thus contributed to the different magnitude of treatment effects in offspring
460 compared to parents. Altogether, experiments in indoor and outdoor conditions showed
461 overall similar direction in plant responses, supporting the ecological importance of
462 environmental predictability in driving variation of plant populations (Johnson et al., 2008).

Does drought intensify the effects of parental and offspring precipitation predictability?

Drought (compared to control conditions) led to reduced plant size independently of OP and PP (Table 1; Fig. 3A–C). This finding is in line with widely accepted results that water availability is crucial for plant growth (e.g., Maggio et al., 2018; Robbins and Dinneny, 2018; Metz et al., 2020).

Our fourth hypothesis was only partly supported. First, drought did not significantly modify the effects of OP on any trait (OP × D: all $P \geq 0.33$; Table 1), although differences between L_o and M_o in total biomass and SRL were marginally significant under drought but not under control conditions (Fig. 3B, D). Second, drought modulated the combined effects of PP and OP on chlorophyll content, which increased significantly under drought compared to control conditions only in L_p–L_o offspring (Fig. S2). This suggests that drought modulates the effects of environmental predictability across generations for specific physiological traits, which is congruent with a study manipulating environmental predictability and thermal shock in *Drosophila mojavensis* (Diaz et al., 2021).

Furthermore, the fact that under drought and more predictable precipitation offspring plants tended to have lower total biomass (Fig. 3B) but higher SRL, *i.e.*, more root length per unit of root biomass (Fig. 3D), suggests that plants changed the functional strategy of water uptake (Müllers et al., 2023). Soil drying is vertically non-uniform, with shallow soil layers drying first and deeper soil layers drying later (Markesteijn et al., 2010). More constant precipitation usually provides water to deeper soil layers. This suggests that producing relatively long roots for a given root biomass may have served to reach accumulated soil humidity in deeper soil layers, and the reduced total biomass (Fig. 3B) suggests that this came at the cost of immediate yield (*i.e.*, biomass production; Müllers et al., 2023).

Despite smaller plant size under drought (Fig. 3A–C), chlorophyll content did not decrease significantly under drought in any PP × OP combination and it even increased in L_p–L_o offspring (Fig. S2). Different studies have shown that the chlorophyll content in most plants decreases under drought stress and that chlorophyll content is only maintained in plants with high drought tolerance (e.g., Li et al., 2006; Jedmowski et al., 2013; Monteoliva et al., 2021). The maintenance of chlorophyll content under drought may therefore indicate increased resilience to drought (Rahdari et al., 2012). Considering both the morphological and physiological trait responses, our findings suggest that drought interacts with environmental predictability mainly through physiological rather than morphological responses. The severity of the water shortage may nevertheless determine which response is expressed, since in an indoor experiment on plants raised from seeds from the same field experiment differences between past predictability treatments appeared only under a long-term drought (Springer et al., 2024), whereas here a moderate but sustained reduction in water supply was already sufficient. Finally, because *P. rhoeas* is well adapted to dry environments (Torra et al., 2011; Pérez-Ramos et al., 2019), it remains to be tested whether species with lower drought tolerance show similar transgenerational responses to precipitation predictability.

Conclusion

With increasing global temperatures, precipitation is rapidly becoming less predictable. Understanding how plant populations will respond to such changes, both within and across generations, will be crucial to forecast their future persistence. Here, we assessed whether differences in precipitation predictability experienced by parental plants shape the phenotype of their offspring, and whether drought modifies that response. Our study shows that parental exposure to less predictable precipitation leaves a phenotypic imprint on the offspring, even

though the parental generation itself showed no detectable phenotypic response to the treatment. This imprint was expressed mainly in combination with the predictability experienced by the offspring and, for chlorophyll content, also in combination with drought. An environmental match across generations was advantageous only following a less predictable parental environment. This suggests that parental effects may confer an advantage primarily when conditions are less predictable, rather than a broad pre-adaptation to the parental environment itself. This interpretation is supported by previous studies conducted on the parental generations, which showed that less predictable precipitation is not necessarily detrimental for plants and may rather favour rapid responses to environmental change. Overall, our study shows that precipitation predictability acts simultaneously within and across generations, and that experiments combining several environmental factors, and exposing offspring to parental as well as to non-parental conditions, are needed to capture the magnitude and direction of parental environmental effects.

Acknowledgements

We thank Instituto Pirenaico de Ecología (IPE-CSIC) and Museo Nacional de Ciencias Naturales (MNCN-CSIC) as host institutions of the project that provided the original seed material, and the Wissenschaftsgarten of Goethe University Frankfurt and its staff, as well as Lutz Stübing and Silas Buese, for providing space, materials and support for the greenhouse experiment.

Funding

This work was supported by the Spanish Ministry of Economy and Competitiveness (grants CGL2012-32459 and CGL2016-76918 to PSF), the Spanish Ministry of Science, Innovation

and Universities (grant PID2024-161670NA-I00 to MMS) and the Swiss National Science Foundation (grants PP00P3_128375 and PP00P3_152929/1 to PSF).

Conflict of interest

All authors declare there are no conflicts of interest.

Author contributions

MMS with PSF designed and MMS conducted the field experiment that gave rise to the original seed material and data on the parental generation. PSF provided the funds and means for the field work, JFS for the greenhouse experiment, and MMS for the data analyses and drafting of the manuscript. LE, CM, JFS and MMS designed the greenhouse experiment. LE and CM conducted the greenhouse experiment with inputs from VH, JFS and MMS. MMS with LL analysed the data, MMS wrote the first draft of the manuscript, and all authors contributed to revisions.

Data availability statement

Data and R code supporting the findings of this study will be archived in the figshare repository upon acceptance of the manuscript for publication.

References

Badyaev, A.V. (2008). Maternal effects as generators of evolutionary change: a reassessment. *Annals of the New York Academy of Sciences*, *1133*(1), 151-161.

Baker, B. H., Sultan, S. E., Lopez-Ichikawa, M., & Waterman, R. (2019). Transgenerational effects of parental light environment on progeny competitive performance and lifetime fitness. *Philosophical Transactions of the Royal Society B*: Biological Sciences, *374*(1768), 20180182.

Bantis, F., Früchtenicht, E., Graap, J., Ströll, S., Reininger, N., Schäfer, L., ... & Brüggemann, W. (2020). The JIP-test as a tool for forestry in times of climate change. *Photosynthetica*, 58, 224-236.

Bates, D., Mächler, M., Bolker, B.M., & Walker, S.C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Bossdorf, O., Shuja, Z., & Banta, J.A. (2009). Genotype and maternal environment affect belowground interactions between *Arabidopsis thaliana* and its competitors. *Oikos*, *118*, 1541–1551.

Burgess, S.C., & Marshall, D.J. (2014). Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos*, *123*(7), 769–776.

Chapin, F.S., Rincon, E., & Huante, P. (1993). Environmental responses of plants and ecosystems as predictors of the impact of global change. *Journal of Biosciences*, *18*, 515-524.

567 Craine, J.M., Froehle, J., Tilman, D.G., Wedin, D.A., & Chapin, III, F.S. (2001). The
568 relationships among root and leaf traits of 76 grassland species and relative abundance
569 along fertility and disturbance gradients. *Oikos*, *93*(2), 274-285.

570 Diaz, F., Kuijper, B., Hoyle, R.B., Talamantes, N., Coleman, J.M., & Matzkin, L.M. (2021).
571 Environmental predictability drives adaptive within- and transgenerational plasticity of
572 heat tolerance across life stages and climatic regions. *Functional Ecology*, *35*(1), 153-
573 166.

574 Fox, J., & Weisberg, S. (2019). An R Companion to Applied Regression, 3rd edn. Thousand
575 Oaks, CA: Sage.

576 Franch-Gras, L., Garcia-Roger, E.M., Serra, M., & Carmona, M.J. (2017). Adaptation in
577 response to environmental unpredictability. *Proceedings of the Royal Society B:*
578 *Biological Sciences*, *284*(1868), 20170427.

579 Galloway, L.F., & Etterson, J.R. (2007). Transgenerational plasticity is adaptive in the wild.
580 *Science*, *318*, 1134–1136.

581 Groot M.P., Kubisch A., Ouborg N.J., Pagel J., Schmid K.J., Vergeer P., & Lampei C. (2017).
582 Transgenerational effects of mild heat in *Arabidopsis thaliana* show strong genotype
583 specificity that is explained by climate at origin. *New Phytologist*, *215*, 1221–1234.

584 Hajek, O.L., & Knapp, A.K. (2022). Shifting seasonal patterns of water availability:
585 ecosystem responses to an unappreciated dimension of climate change. *New*
586 *Phytologist*, *233*(1), 119-125.

587 Herman J.J., & Sultan S.E. (2011). Adaptive transgenerational plasticity in plants: case
588 studies, mechanisms, and implications for natural populations. *Frontiers in Plant Science*,
589 2, 102.

590 Hilker, M., & Schmülling, T. (2019). Stress priming, memory, and signalling in plants. *Plant,*
591 *Cell & Environment*, 42(3), 753–761.

592 IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II
593 and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change
594 [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland,
595 151 pp.

596 Jedmowski, C., Ashoub, A., & Brüggemann, W. (2013). Reactions of Egyptian landraces of
597 *Hordeum vulgare* and *Sorghum bicolor* to drought stress, evaluated by the OJIP
598 fluorescence transient analysis. *Acta Physiologiae Plantarum*, *35*, 345-354.

599 Johnson, M.T., Dinnage, R., Zhou, A.Y., & Hunter, M.D. (2008). Environmental variation
600 has stronger effects than plant genotype on competition among plant species. *Journal of*
601 *Ecology*, 96(5), 947-955.

602 Lampei, C. (2019). Multiple simultaneous treatments change plant response from adaptive
603 parental effects to within-generation plasticity, in *Arabidopsis thaliana*. *Oikos*, *128*(3),
604 368–379.

605 Latzel, V., Klimešová, J., Hájek, T., Gómez, S., & Šmilauer, P. (2010). Maternal effects alter
606 progeny's response to disturbance and nutrients in two Plantago species. *Oiko*s, *119*(11),
607 1700–1710.

608 Latzel, V., Fischer, M., Groot, M., Gutzat, R., Lampei, C., Ouborg, J., ... & Bossdorf, O.
609 (2023). Parental environmental effects are common and strong, but unpredictable, in
610 *Arabidopsis thaliana*. *New Phytologist*, *237*(3), 1014–1023.

611 Lawrence, M.J. (1975). The genetics of self-incompatibility in *Papaver rhoeas*. Proceedings
612 of the Royal Society of London B, 188, 275–285.

613 Lenth, R.V. (2016). Least-Squares Means: The *R* Package lsmeans. *Journal of Statistical*
614 *Software*, *69*(1), 1–33.
615 Leung, C., Rescan, M., Grulois, D., & Chevin, L. (2020). Reduced phenotypic plasticity
616 evolves in less predictable environments. *Ecology Letters*, *23*(11), 1664–1672.
617 Li, R.H., Guo, P.G., Baum, M., Grando, S., & Ceccarelli, S. (2006). Evaluation of chlorophyll
618 content and fluorescence parameters as indicators of drought tolerance in barley.
619 *Agricultural Sciences in China*, *5*(10), 751-757.
620 Maggio, A., Bressan, R.A., Zhao, Y., Park, J., & Yun, D.J. (2018). It's hard to avoid
621 avoidance: Uncoupling the evolutionary connection between plant growth, productivity
622 and stress "tolerance". *International Journal of Molecular Sciences*, *19*(11), 3671.
623 March-Salas, M., & Fitze, P.S. (2019a). A multi-year experiment shows that lower
624 precipitation predictability encourages plants' early life stages and enhances population
625 viability. *PeerJ*, *7*, e6443.
626 March-Salas, M., & Fitze, P.S. (2019b). Changes in environmental predictability alter a
627 plant's chemical composition and associated ecosystem services. *Environmental and*
628 *Experimental Botany*, *168*, 103865.
629 March-Salas, M., van Kleunen, M., & Fitze, P.S. (2019). Rapid and positive responses of
630 plants to lower precipitation predictability. *Proceedings of the Royal Society B:*
631 *Biological Sciences*, *286*(1913), 20191486.
632 March-Salas, M., Fandos, G., & Fitze, P.S. (2021a). Effects of intrinsic environmental
633 predictability on intra-individual and intra-population variability of plant reproductive
634 traits and eco-evolutionary consequences. *Annals of Botany*, *127*(4), 413–423.
635 March-Salas, M., van Kleunen, M., & Fitze, P.S. (2021b). Effects of intrinsic precipitation-
636 predictability on root traits, allocation strategies and the selective regimes acting on them.
637 *Oikos*, *2022*(1), oik.07970.

March-Salas, M., Scheepens, J.F., van Kleunen, M., & Fitze, P.S. (2022). Precipitation predictability affects intra- and trans-generational plasticity and causes differential selection on root traits of *Papaver rhoeas*. *Frontiers in Plant Science*, *13*, 998169.

Markesteijn, L., Iraipi, J., Bongers, F., & Poorter, L. (2010). Seasonal variation in soil and plant water potentials in a Bolivian tropical moist and dry forest. *Journal of Tropical Ecology*, *26*(5), 497-508.

Marshall, D.J., & Uller, T. (2007). When is a maternal effect adaptive? *Oikos*, *116*(12), 1957-1963.

Matsubara, S. (2018). Growing plants in fluctuating environments: why bother? *Journal of Experimental Botany*, *69*(20), 4651-4654.

Mazer, S. J., & Gorchov, D. L. (1996). Parental effects on progeny phenotype in plants: distinguishing genetic and environmental causes. *Evolution*, *50*(1), 44-53.

McNaughton, I. H., & Harper, J. L. (1964). Papaver L. *Journal of Ecology*, *52*(3), 767–793.

Metz, J., Lampei, C., Bäumler, L., Bocherens, H., Dittberner, H., Henneberg, L., de Meaux, J., & Tielbörger, K. (2020). Rapid adaptive evolution to drought in a subset of plant traits in a large-scale climate change experiment. *Ecology Letters*, *23*(11), 1643–1653.

Monteoliva, M.I., Guzzo, M.C., & Posada, G.A. (2021). Breeding for drought tolerance by monitoring chlorophyll content. *Gene Technol*, *10*(3), 1-11.

Müllers, Y., Postma, J. A., Poorter, H., & van Dusschoten, D. (2023). Deep-water uptake under drought improved due to locally increased root conductivity in maize, but not in faba bean. *Plant, Cell & Environment*, *46*(7), 2046-2060.

Muñoz-Huerta, R.F., Guevara-Gonzalez, R.G., Contreras-Medina, L.M., Torres-Pacheco, I., Prado-Olivarez, J., & Ocampo-Velazquez, R.V. (2013). A review of methods for sensing

the nitrogen status in plants: advantages, disadvantages and recent advances. *Sensors*, *13*(8), 10823-10843.

Münzbergová, Z., Hadincová, V., Skálová, H., & Vandvik, V. (2017). Genetic differentiation and plasticity interact along temperature and precipitation gradients to determine plant performance under climate change. *Journal of Ecology*, *105*(5), 1358-1373.

Pennekamp, F., Iles, A.C., Garland, J., Brennan, G., Brose, U., Gaedke, U., Jacob, U., Kratina, P., Matthews, B., Munch, S., Novak, M., Palamara, G.M., Rall, B.C., Rosenbaum, B., Tabi, A., Ward, C., Williams, R., Ye, H., & Petchey, O.L. (2019). The intrinsic predictability of ecological time series and its potential to guide forecasting. *Ecological Monographs*, *89*(2), e01359.

Pérez-Ramos, I. M., Matías, L., Gómez-Aparicio, L., & Godoy, Ó. (2019). Functional traits and phenotypic plasticity modulate species coexistence across contrasting climatic conditions. *Nature Communications*, *10*(1), 2555.

Poorter, H., Fiorani, F., Pieruschka, R., Wojciechowski, T., van der Putten, W.H., Kleyer, M., Schurr, U., & Postma, J. (2016). Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytologist*, 212(4), 838–855.

Proulx, S.R., & Teotónio, H. (2017). What kind of maternal effects can be selected for in fluctuating environments? *American Naturalist*, *189*(6), E118–E137.

Puy, J., Carmona, C.P., Dvořáková, H., Latzel, V., & de Bello, F. (2021). Diversity of parental environments increases phenotypic variation in Arabidopsis populations more than genetic diversity but similarly affects productivity. *Annals of Botany*, *127*(4), 425-436.

R Core Team. (2020). R: A language and environment for statistical computing. Vienna, Austria. URL: https://www.R-project.org/

Rahdari, P., Hosseini, S.M., & Tavakoli, S. (2012). The studying effect of drought stress on germination, proline, sugar, lipid, protein and chlorophyll content in purslane (*Portulaca oleracea* L.) leaves. *Journal of Medicinal Plants Research*, *6*(9), 1539-1547.

Reed, T.E., Waples, R.S., Schindler, D.E., Hard, J.J., & Kinnison, M.T. (2010). Phenotypic plasticity and population viability: the importance of environmental predictability. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1699), 3391-3400.

Ren, G., Yang, B., Cui, M., Yu, H., Fan, X., Dai, Z., Sun, J., Li, G., Zhang, H. & Du, D. (2022) Additive effects of warming and nitrogen addition on the performance and competitiveness of invasive *Solidago canadensis* L. *Frontiers in Plant Science*, *13*, 1017554.

Roach, D.A., & Wulff, R.D. (1987). Maternal effects in plants. *Annual Review of Ecology and Systematics*, 18, 209–235.

Robbins, N. E., & Dinneny, J. R. (2018). Growth is required for perception of water availability to pattern root branches in plants. *Proceedings of the National Academy of Sciences*, *115*(4), E822-E831.

Springer, K., Coquery, T., Holland, V., Fitze, P.S., Scheepens, J.F., & March-Salas, M. (2024). Precipitation predictability drives evolution of drought strategies in the common poppy, *Papaver rhoeas*. *Functional Ecology*, 38(1), 233–244. https://doi.org/10.1111/1365-2435.14456

706 Springer, K., Fitze, P. S., Laccetti, L., Mercé, G., & March-Salas, M. (2026). Precipitation
707 predictability shapes plant growth trajectories, climate sensitivity, and fitness pathways
708 within and across generations. *EcoEvoRxiv*. https://doi.org/10.32942/X2PQ36

709 Strasser, R. J., Srivastava, A., & Tsimilli-Michael, M. (2000). The fluorescence transient as a
710 tool to characterize and screen photosynthetic samples. In M. Yunus, U. Pathre, & P.
711 Mohanty (Eds.), Probing Photosynthesis: Mechanisms, Regulation and Adaptation (pp.
712 445–483). London: Taylor & Francis.

713 Strasser, R. J., Tsimilli-Michael, M., & Srivastava, A. (2004). Analysis of the chlorophyll a
714 fluorescence transient. In Chlorophyll a fluorescence: a signature of photosynthesis (pp.
715 321–362). Dordrecht: Springer Netherlands.

716 Torra, J., Royo Esnal, A., & Recasens Guinjuan, J. (2011). Management of herbicide-resistant
717 *Papaver rhoeas* in dry land cereal fields. *Agronomy for Sustainable Development*, *31*(3),
718 483–490.

719 Tutin, T.G., Halliday, G., & Beadle, M. (1993). *Flora Europaea*. Cambridge University Press.

720 Uller, T., Nakagawa, S. & English, S. (2013). Weak evidence for anticipatory parental effects
721 in plants and animals. *Journal of Evolutionary Biology*, *26*, 2161–2170.

722 von Wangenheim, D., Banda, J., Schmitz, A., Boland, J., Bishopp, A., Maizel, A., ... &
723 Bennett, M. (2020). Early developmental plasticity of lateral roots in response to
724 asymmetric water availability. *Nature Plants*, *6*(2), 73-77.

725 Wickham H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New
726 York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org

727 Wu, X., Bellagio, T., Peng, Y., Czech, L., Lin, M., Lang, P., ... & Exposito-Alonso, M.
728 (2026). Rapid adaptation and extinction in synchronized outdoor evolution experiments
729 of Arabidopsis. *Science*, *391*(6792), eadz0777.
730 Yamauchi, T., Pedersen, O., Nakazono, M., & Tsutsumi, N. (2021). Key root traits of Poaceae
731 for adaptation to soil water gradients. *New Phytologist*, 229(6), 3133–3140.
732 Yin, J., Lin, X., Yao, J., Li, Q.Q., & Zhang, Y. (2022). Genotypic variation of
733 transgenerational plasticity can be explained by environmental predictability at origins.
734 *Oikos*, *2022*(5).
735 Zhou, M., Wang, J., Bai, W., Zhang, Y., & Zhang, W.H. (2019). The response of root traits to
736 precipitation change of herbaceous species in temperate steppes. *Functional Ecology*, *33*
737 (10), 2030–2041.

738 **Figure legends**

739 **Figure 1:** Interaction between parental (PP) and offspring (OP) precipitation predictability on
740 *Papaver rhoeas* phenotypes. The figure shows the offspring plant response to OP, while PP is
741 represented on the x-axis. Means ± standard errors (SE) of the measured traits are shown: (A)
742 growth rate, (B) total biomass, (C) length of the longest leaf (leaf length), (D) Specific Root
743 Length, (E) relative leaf chlorophyll content, and (F) Performance Index (photosynthetic
744 performance). Red and blue colours indicate less (L_o) and more (M_o) predictable OP,
745 respectively. Post-hoc contrasts among all treatment combinations (Tukey's tests on the
746 model estimates) are indicated with letters; they are shown in all panels to allow comparison
747 among treatment combinations, also where the underlying interaction is not significant. The
748 significance of the corresponding model terms (PP, OP and PP × OP) is given in each panel
749 (· $0.1 > P \geq 0.05$; * $0.05 > P \geq 0.01$; ** $0.01 > P \geq 0.001$; *** $P < 0.001$; NS $P \geq 0.1$; for
750 details see Table 1). For relative leaf chlorophyll content (E), the three-way PP × OP ×
751 drought interaction was also significant, so the lower-order terms are not interpreted (Table
752 1); the corresponding contrasts are shown in Fig. S2. Sample size was 120, and effect sizes for
753 significant main treatment effects are included in Table S1.

754 **Figure 2:** Effects of precipitation predictability on the parental and offspring generation. The
755 figure shows the phenotypic response to precipitation predictability of the parental generation
756 in an outdoor experiment in Jaca (Spain) (left column), and of the offspring generation in an
757 indoor greenhouse experiment in Frankfurt am Main (Germany) (right column). Means ±
758 standard errors (SE) are shown for growth rate (A, D), total biomass (B, E) and Specific Root
759 Length (C, F). Blue and red colours indicate more or less predictable precipitation,
760 respectively. Significant differences between both predictability treatments in each generation

761 are shown (** $0.01 > P \geq 0.001$; * $0.05 > P \geq 0.01$; NS $P \geq 0.1$). Sample size was 66, and
762 effect sizes for significant main treatment effects are included in Table S1.

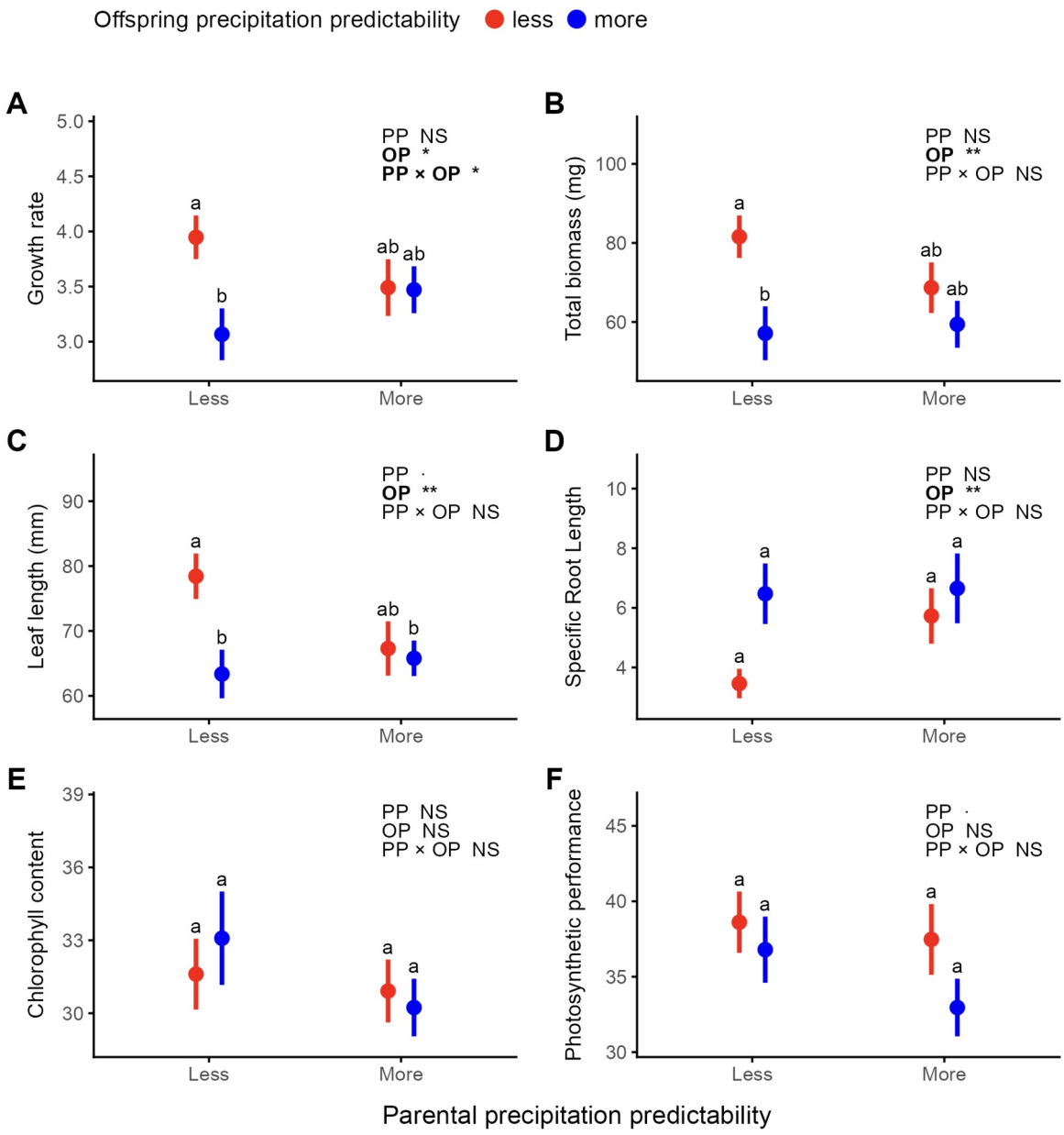
763 **Figure 3:** Effects of the drought treatment (D) on the offspring response to offspring
764 precipitation predictability (OP). The figure shows the offspring plant response to OP, while
765 the control and drought levels are represented on the x-axis. Means ± standard errors (SE) of
766 the measured traits are shown: (A) growth rate, (B) total biomass, (C) length of the longest
767 leaf (leaf length), (D) Specific Root Length, (E) relative leaf chlorophyll content, and (F)
768 Performance Index (photosynthetic performance). Blue and red colours indicate more (M_o)
769 and less (L_o) predictable OP, respectively. Post-hoc contrasts among all treatment
770 combinations (Tukey's tests on the model estimates) are indicated with letters; they are shown
771 in all panels to allow comparison among treatment combinations, also where the underlying
772 interaction is not significant. The significance of the corresponding model terms (OP, D and
773 OP × D) is given in each panel (* $0.05 > P \geq 0.01$; ** $0.01 > P \geq 0.001$; *** $P < 0.001$; NS P
774 ≥ 0.1; for details see Table 1). For relative leaf chlorophyll content (E), the three-way
775 interaction between parental precipitation predictability, OP and D was also significant, so the
776 lower-order terms are not interpreted (Table 1); the corresponding contrasts are shown in Fig.
777 S2. Sample size was 120, and effect sizes for significant main treatment effects are included
778 in Table S1.

Table and Figures:

Table 1. Effect of parental precipitation predictability (PP), offspring precipitation predictability (OP) and drought (D) on the morphological and physiological response of the offspring. Response variables are rosette diameter growth rate (in mm/day), total biomass (in mg), length of the longest leaf (leaf length; in mm), Specific Root Length (SRL, in cm/mg), relative leaf chlorophyll content, and Performance Index (PI_{abs}; photosynthetic performance). Linear Mixed-effects Models (LMMs) included initial rosette diameter as covariate, and PP, OP, D, and their two- and three-way interactions as fixed terms. Transformations applied to the response variable are indicated. Significance is shown as * $0.05 > P \geq 0.01$; ** $0.01 > P \geq 0.001$; *** $P < 0.001$, and • reflects marginal effects ($0.1 > P \geq 0.05$). Sample size was 120.

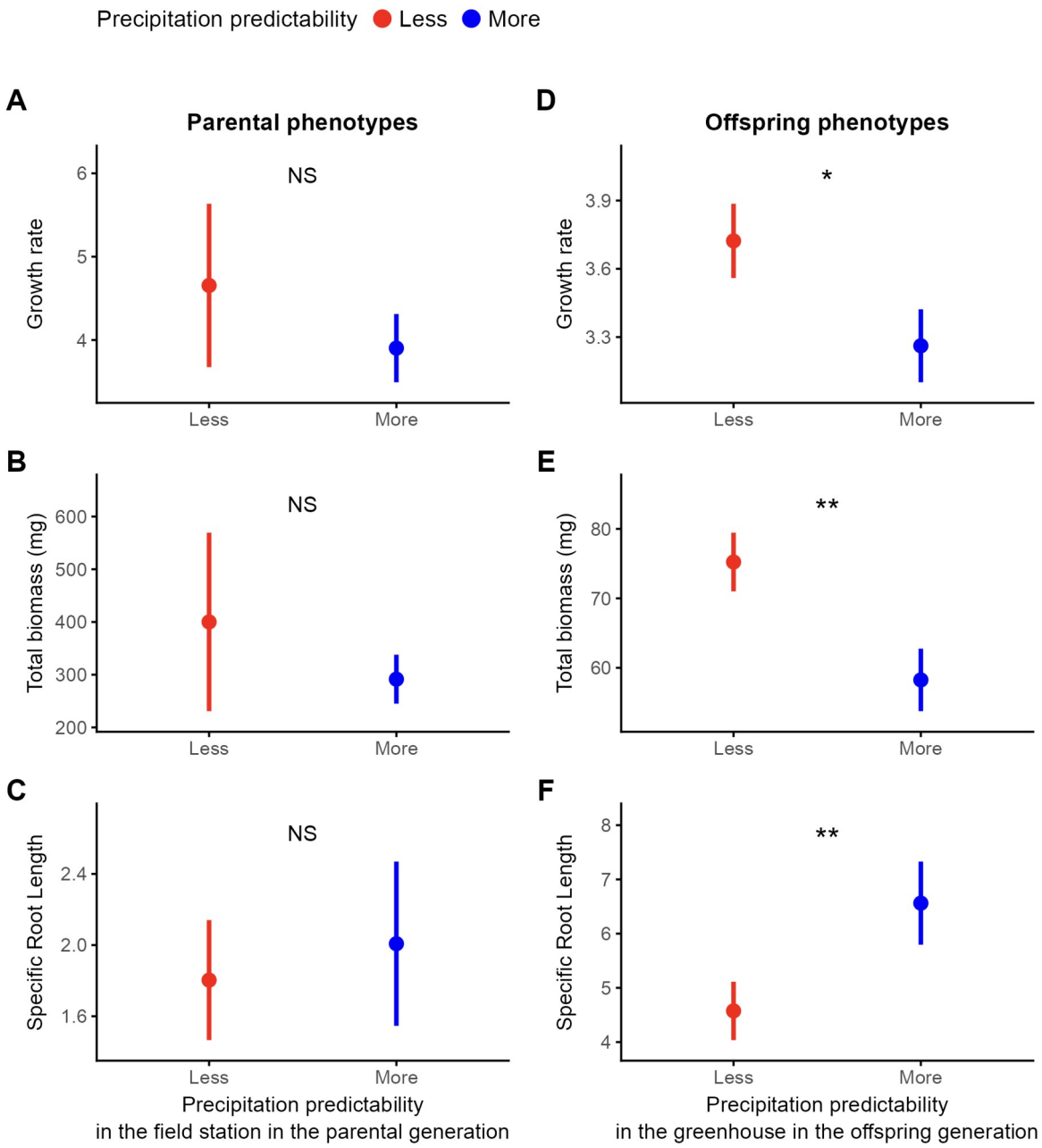
		Growth rate [a]			Total biomass			Leaf length [b]		
Parameter	**df**	χ^2	***P-value***		χ^2	***P-value***		χ^2	***P-value***	
Initial diameter	1	0.219	0.639		12.811	<0.001	***	16.578	<0.001	***
PP	1	0.099	0.753		1.659	0.198		3.273	0.070	•
OP	1	3.960	0.047	*	8.676	0.003	**	7.835	0.005	**
D	1	9.999	0.002	**	17.602	<0.001	***	13.750	<0.001	***
PP × OP	1	4.304	0.038	*	1.316	0.251		2.418	0.120	
PP × D	1	0.592	0.441		0.072	0.789		0.986	0.321	
OP × D	1	0.206	0.650		0.389	0.533		0.131	0.718	
PP × OP × D	1	0.968	0.325		1.098	0.295		0.009	0.926	
		SRL [c]			**Chlorophyll content** [c]			**Photosynthetic performance** [c]		
Parameter	**df**	χ^2	***P-value***		χ^2	***P-value***		χ^2	***P-value***	
Initial diameter	1	13.148	<0.001	***	0.050	0.824		7.896	0.005	**
PP	1	1.535	0.215		0.718	0.397		3.495	0.062	•
OP	1	7.255	0.007	**	0.114	0.735		1.877	0.171	
D	1	17.460	<0.001	***	4.810	0.028	*	0.037	0.847	
PP × OP	1	0.967	0.325		0.490	0.484		0.532	0.466	
PP × D	1	1.579	0.209		1.505	0.220		0.173	0.678	
OP × D	1	0.939	0.332		0.837	0.360		0.750	0.387	
PP × OP × D	1	0.065	0.798		7.681	0.006	**	0.431	0.512	

transformations: a = $x^{0.5}$; b = x^2; *c* = *log(x)*



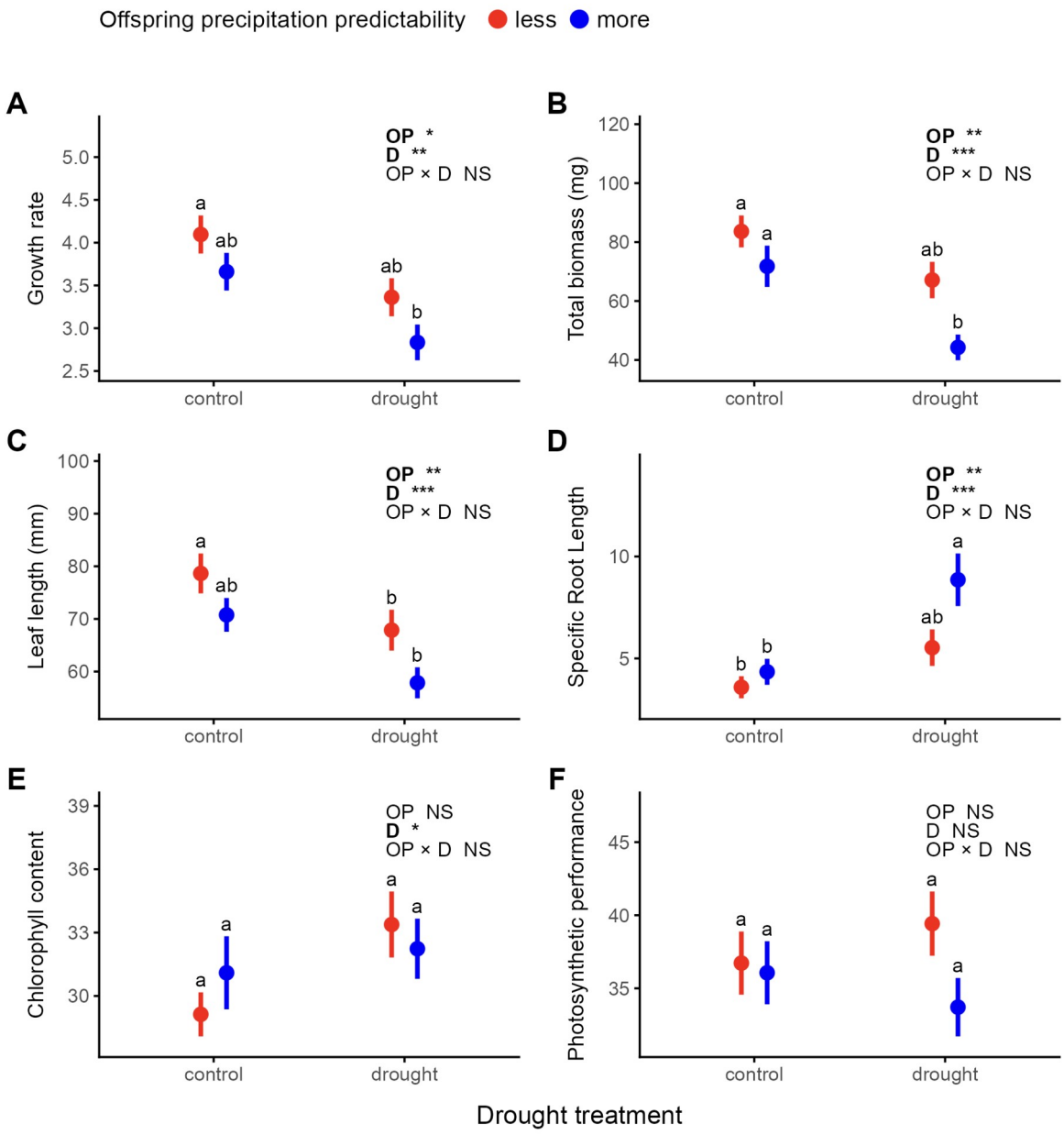
787

788 **Figure 1**



789

790 **Figure 2**



791

792 **Figure 3**

Supporting information for: Drought and parental effects shape plant responses to decreased precipitation predictability

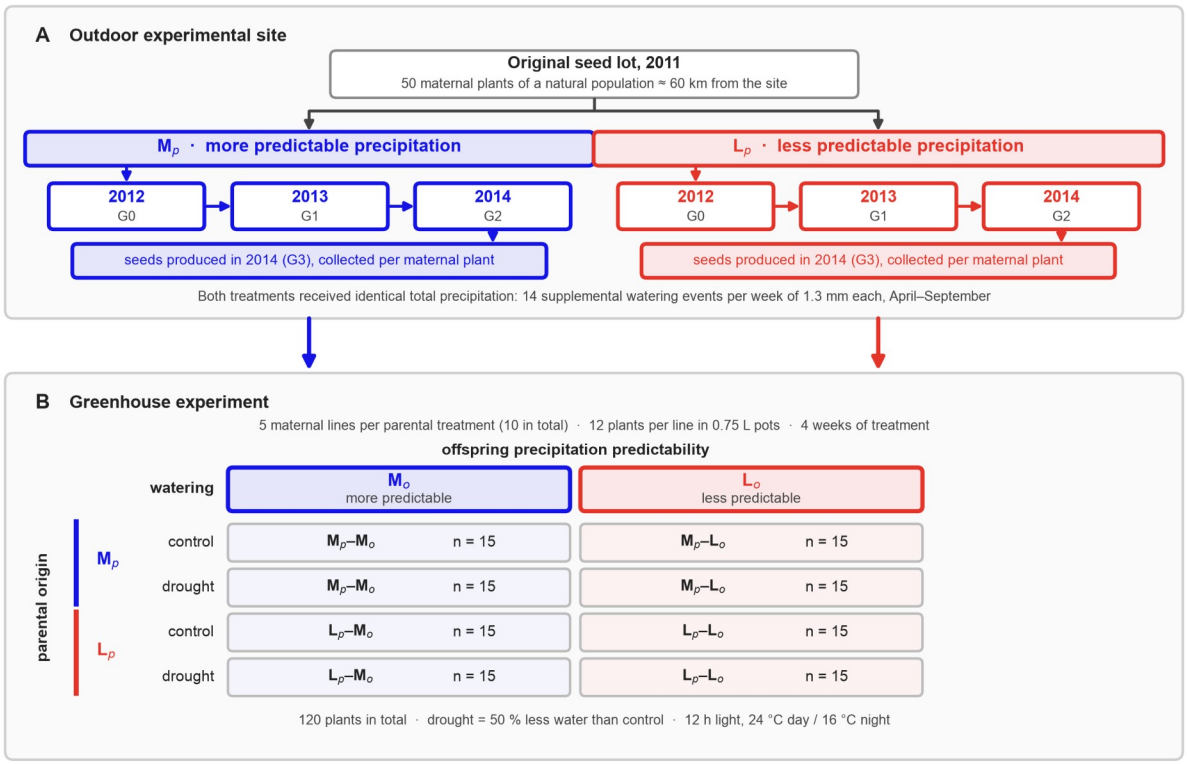


Figure S1: Schematic representation of the experimental design. In the outdoor field experiment at 'El Boalar' (Jaca, Spain), plants were exposed to more (M_p) or to less (L_p) predictable precipitation over three consecutive generations (sown in 2012, 2013 and 2014; four plots of 7.2 m² per treatment). Seeds produced in 2014 by five maternal lines per PP treatment were sown in a greenhouse at Goethe University Frankfurt (Germany), where offspring of both parental treatments were exposed for four weeks to more (M_o) or to less (L_o) predictable precipitation, crossed with control or drought (50% less water) conditions. This resulted in eight parental × offspring predictability × drought combinations and 120 plants in total (ten seed families, twelve plants each, three plants per seed family and combination).

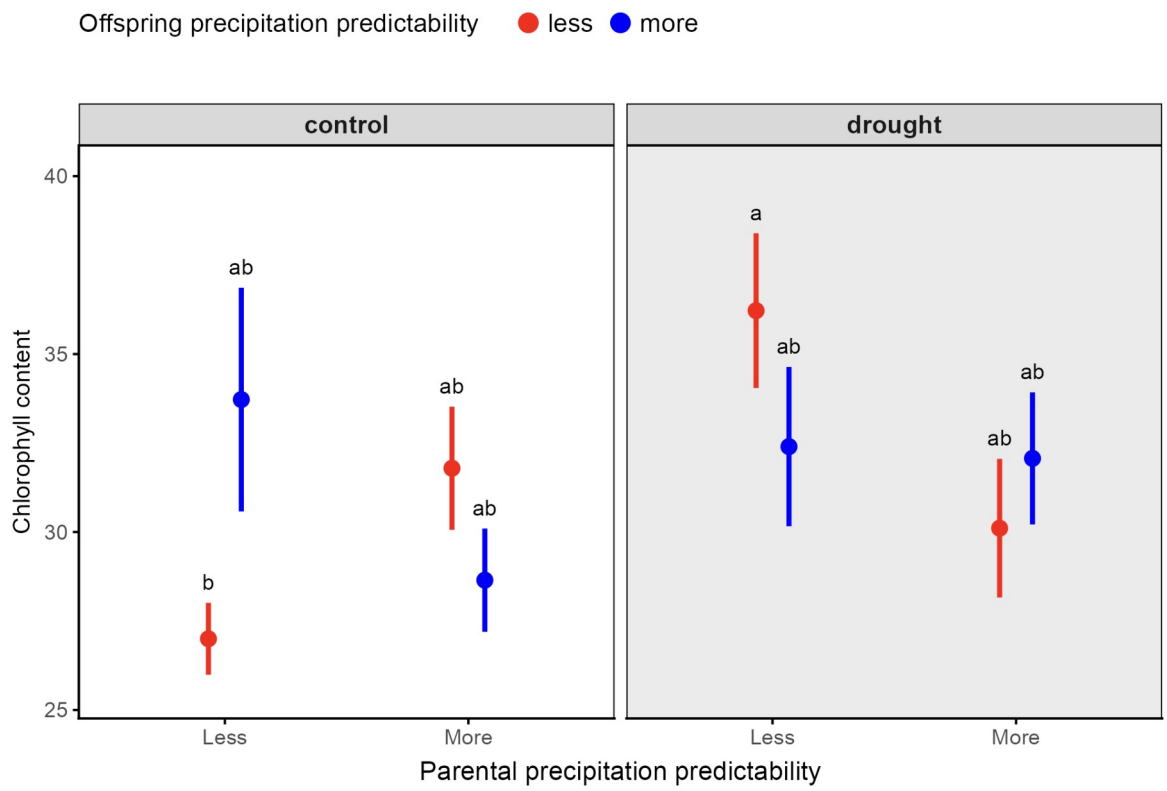


Figure S2: Significant three-way interaction between the drought treatment (D), parental precipitation predictability (PP) and offspring precipitation predictability (OP) on the relative leaf chlorophyll content of *Papaver rhoeas***.** Means ± standard errors (SE) are shown. Blue and red colours indicate more (M_o) and less (L_o) predictable OP, respectively. PP is represented on the x-axis; control conditions are shown in the left panel and drought conditions in the right panel (grey background). Post-hoc contrasts among all treatment combinations (Tukey's tests on the model estimates) are indicated with letters. Sample size was 110.

Table S1: Effect sizes of the treatments on offspring traits. Values are differences between estimated marginal means on the original scale, with 95% confidence intervals, from the models in Table 1. Main effects are averaged over the levels of the other factors; for traits with significant interactions (growth rate: PP × OP; chlorophyll content: PP × OP × D), the simple effects within each PP treatment or the contrasts in Fig. S2 are the relevant estimates. Significant treatment (OP, D, PP) effects are bolded.

Trait (unit)	**OP: $L_o - M_o$**	**D: drought − control**	**PP: $L_p - M_p$**	**$L_o - M_o$ within L_p**	**$L_o - M_o$ within M_p**
Growth rate (mm/day)	**0.49 [−0.13, 1.11]**	**−0.79 [−1.41, −0.17]**	0.08 [−1.57, 1.73]	1.01 [0.07, 1.96]	−0.03 [−0.86, 0.79]
Total biomass (mg)	**16.0 [1.9, 30.2]**	**−22.7 [−36.8, −8.7]**	9.3 [−31.4, 49.9]	22.3 [0.9, 43.7]	9.8 [−9.0, 28.5]
Leaf length (mm)	**8.4 [2.0, 14.7]**	**−11.2 [−17.6, −4.9]**	5.9 [−1.8, 13.5]	12.7 [4.1, 21.2]	3.7 [−5.7, 13.2]
SRL (cm/mg)	**−1.33 [−2.43, −0.24]**	**2.11 [0.95, 3.26]**	−0.89 [−2.61, 0.83]	−1.64 [−3.07, −0.21]	−0.94 [−2.63, 0.75]
Chlorophyll content (SPAD units)	**−0.3 [−2.9, 2.4]**	**2.4 [−0.2, 5.1]**	1.4 [−3.1, 5.9]	−1.1 [−4.8, 2.7]	0.5 [−3.2, 4.2]
Photosynthetic performance (dimensionless)	2.6 [−1.9, 7.1]	−0.3 [−4.8, 4.2]	3.5 [−11.0, 17.9]	1.3 [−5.5, 8.2]	3.7 [−2.1, 9.6]