1   **Precipitation predictability drives evolution of drought tolerance in *Papaver rhoeas***

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Abstract

Current climate change leads to an increase in the frequency and intensity of droughts and to a decreased precipitation predictability. The few studies investigating plant evolutionary responses to contrasting predictability regimes showed that intrinsic precipitation predictability shapes plant phenotypic variation, drives evolution of phenotypic plasticity, and can vary in strength and direction of selection. This suggests that the selection pressure induced by decreased precipitation predictability may lead to plants coping better with severe drought events. To investigate this unsolved question, we performed a common-garden experiment applying control, short-term and long-term drought treatments on seedlings of *Papaver rhoeas* (Papaveraceae) whose progenitors experienced less *versus* more precipitation predictability treatments for three consecutive generations. In addition, to assess whether competition modulates plant responses, half of the plants were grown together with the widespread herbaceous plant *Galium album* (Rubiaceae). In the presence of long drought episodes, plants whose progenitors experienced less predictable precipitation survived longer than those whose progenitors experienced more predictable precipitation. Furthermore, plants whose progenitors experienced less predictable precipitation had lower biomass, which is likely to reduce water loss via transpiration, and, across all drought treatments, they showed lower root investment. Altogether our results indicate that lower precipitation predictability promotes drought tolerance rather than a drought-avoidance strategy to their offspring. Overall, our experiment highlights that precipitation predictability is an important driver of plant evolution, potentially shifting evolutionary trajectories of plants under increasing intensity of drought events.

Introduction

Current climate change leads to increased global temperatures, higher frequency and intensity of drought events, as well as to decreased precipitation predictability (Pörtner et al., 2022; Swain et al., 2018; Xu et al., 2020). Drought events are caused by changes in mean precipitation and lead to water scarcity (Dai, 2013), while changes in precipitation predictability are defined as the changes in the degree of temporal correlation between precipitation events (Ashander et al., 2016) and affect the temporal availability of water (Karl et al., 1995). Although both climatic factors are independent, drought and changes in precipitation predictability can occur simultaneously (Singh et al., 2018), with exacerbated effects for plant populations. Consequently, plants should continuously and rapidly adapt to these changing environmental conditions.

Drought hinders plant performance and usually increases mortality rates, reducing the persistence of plant populations (Maggio et al., 2018). Plants can successfully overcome unfavourable drought conditions by increased plasticity (Hoffmann & Sgrò, 2011; Lambrecht et al., 2020). They can also use a drought-escape, a drought-avoidance or a drought-tolerance strategy in order to deal with reduced precipitation (Bowles et al., 2021; Levitt, 1980; Rauschkolb et al., 2022). This would require rapid phenological, morphological or physiological adjustments. For instance, they can flower earlier to escape from drought (Franks et al., 2007; Metz et al., 2020), generate longer roots to take up water from deeper soil layers and thus avoid water deficit (Padilla & Pugnaire, 2007), or reduce water loss by lowering their leaf size or changing leaf water potential in order to tolerate drought (Bartlett et al., 2014; Bowles et al., 2021; Májeková et al., 2019), respectively. However, how environmental conditions during previous generations shape plant strategies to current drought conditions has been little studied.

Environmental predictability can alter life-history traits and phenotypic variation, and can affect the persistence of plant populations (Reed et al., 2010; Ashander et al., 2016; March-Salas et al., 2019; March-Salas & Fitze, 2019a). Experimental studies showed advanced reproduction under less predictable conditions, which increased plant fitness (March-Salas et al., 2019, 2021). Decreased predictability may favour rapid transgenerational responses, increase selection pressure (March-Salas et al. 2019), and it will likely cause evolution of reduced phenotypic plasticity (Leung et al., 2020; Tufto, 2015; Franch-Gras et al., 2017; March-Salas et al., 2022; Yin et al. 2022). Reduced transgenerational plasticity under decreased predictability could favour rapid evolution in adaptive traits as well as adjustments to severe droughts, which may shield a population from selection (Merilä & Hendry, 2014; Metz et al., 2020). This is supported by previous studies that showed that less predictable precipitation can rapidly vary the root functional investment, favouring fitness changes in the next generation if these offspring were subjected to the parental conditions (March-Salas et al., 2022; March-Salas, van Kleunen & Fitze, 2021; Yamauchi et al., 2021; Zhou et al., 2019). Therefore, the degree of precipitation predictability in progenitors can evolve the functional strategy used by plants to deal with climatic stressors such as increasing frequency and intensity of drought conditions. It is then important to investigate whether plants are predisposed by progenitors (either through maternal effects or through selection) and whether they show adaptive changes that allow them to cope with drought events.

In our study, we selected maternal lines of the widespread annual herb *Papaver rhoeas* L. (Papaveraceae) that were experimentally exposed to more and to less predictable precipitation during three consecutive generations. To investigate if plants show changes due to these past precipitation predictability treatments, we sowed seeds of the maternal lines in a common-garden experiment. To assess whether past precipitation predictability led to a specific

84   plant strategy, and whether this strategy benefited them to withstand drought, the offspring of

85   these selected maternal lines were exposed to control, short-term and long-term drought, and

86   wilting time, plant size and root traits were measured. We expect: (i) that different past

87   precipitation predictability treatments led to different phenotypes and to different plasticity,

88   expressed under common-garden conditions; (ii) that evolutionary change was stronger under

89   lower past precipitation predictability, given that progenitors under this treatment showed lower

90   transgenerational trait plasticity in a previous study; and (iii) that lower past precipitation

91   predictability favoured drought stress tolerance rather than drought-avoidance.

92

93   **Materials and methods**

94   **Study species**

95   The annual herb *Papaver rhoeas* L. (common poppy, Papaveraceae) is a cosmopolitan and fast-

96   growing species. It is widespread throughout Europe, over central and northern Asia, North

97   Africa, and the Canary Islands (McNaughton & Harper, 1964). This species is an erect herb,

98   which is usually between 10 and 50 cm high. The leaves are hairy and particularly variable in

99   their shape and size. The basal leaves form a rosette of pinnatipartite and alternate leaves (Tutin

100 et al., 1993). This species has a slender primary tap root with multiple lateral secondary roots

101 (McNaughton & Harper, 1964). *Papaver rhoeas* can withstand dry conditions (Torra et al.,

102 2011), being commonly associated to disturbed habitats, arable lands, and cereal crops, where

103 it occurs frequently with few or no competitors, and it also grows in communities with other

104 herbaceous species (McNaughton & Harper, 1964).

105 **Past precipitation predictability treatments**

106 In 2012, seeds of *P. rhoeas* were sown at the experimental field station 'El Boalar' (42°33'N,

107 0°37'W, 705 m a.s.l.; Jaca, Spain), where the natural intrinsic precipitation predictability was

108 manipulated by means of an automated watering system (March-Salas et al., 2019; March-Salas

109 & Fitze, 2019a). Plants were either exposed to more (M) or to less (L) predictable precipitation

110 (March-Salas et al., 2019; March-Salas et al., 2021). To this end, supplemental watering events

111 at regular time-intervals for the M treatment, and at random time-intervals for the L treatment,

112 were added to the natural precipitation, resulting in a weighted permutation entropy (i.e., a

113 measure that is inversely related to intrinsic predictability; Pennekamp et al., 2019) of 0.77 in

114 M and 0.86 in L. Intrinsic precipitation predictability in M was equal to the one of natural

115 precipitation, and the intrinsic precipitation predictability of L was 11.69% lower than that of

116 M and of the natural precipitation (March-Salas et al., 2019). Each treatment was applied to

117 four plots and both treatments consisted of the same total amount of precipitation (natural +

118 supplemental) and of 14 supplemental watering events per week (each of 1.3 mm). The same

119 watering treatment was applied to each generation from April to the end of September.

120   These treatments were applied over three consecutive generations in such a way that

121 offspring seeds were always sown under the same precipitation predictability treatment, but

122 never in the plot of the maternal plant. Seeds generated in 2012 (G1) were sown in 2013 and

123 raised under the same treatment as their ancestor (G0). The seeds generated in 2013 (G2) were

124 sown in 2014 and raised under the same treatment as their ancestors (G0 and G1). Finally, the

125 seeds generated in 2014 (G3) were collected, separated by maternal plant, and stored in dry and

126 dark conditions.

127   **Experimental setup**

128 In winter 2022-2023, a common-garden experiment with the G3 seeds was conducted in a

129 greenhouse at Goethe University Frankfurt (50°10'N, 8°38'E, Frankfurt am Main, Germany).

130   Six seed families were randomly selected from each predictability treatment and sown in

131   November 2022 in separate trays. This random selection included either one or two seed

132   families from each plot. In December 2022, seedlings from each seed family and predictability

133   treatment were randomly selected and transplanted into 0.75 L pots. To increase the chances of

134   having one alive seedling per pot, we initially transplanted two seedlings from the same seed

135   family and predictability treatment in the middle of each pot and separated by approx. 3 cm.

136   Pots were submitted to three levels of drought treatments and two levels of competition (*see*

137   details below). To have four replicates/pots per seed family and treatment level, a total number

138   of 576 seedlings were transplanted into 288 pots (i.e., six seed families × two predictability

139   treatments × three drought treatments × two competition treatments × four replicates/pots × two

140   seedlings per pot). Of each plant, the largest diameter (in cm) and the number of leaves were

141   measured the day after the transplantation, and used in the analysis to account for potential

142   effects of the initial size of the selected seedlings. The pots were positioned in 12 trays (each

143   tray with 24 pots), and the position of the trays in the greenhouse as well as the position of the

144   24 pots within each tray was randomized once per week. Pots were watered with sufficient

145   water for two weeks, so that the plants could acclimate after the transplantation. After this

146   establishment period, pots with two alive seedlings were randomly thinned to one seedling

147   (hereafter referred to as day 1).

148   From day 1 and for four weeks, seedlings were exposed to long-term drought, short-

149   term drought or to a control treatment (hereafter referred as 'drought treatments'). Pots under

150   the long-term drought treatment did not receive any water for the four weeks. Pots under the

151   short-term drought treatment received sufficient water during the first week and were not

152   watered during the next three weeks. The control pots received sufficient water during the four

153   weeks. Each drought treatment was applied to one third of the pots (i.e., 96 pots within four

154   trays per drought treatment). The average soil humidity of each treatment was measured in three

155   pots per tray (i.e., 12 pots per drought treatment) using soil moisture sensors (ML3 ThetaProbe,

156   Delta-T Devices Ltd, Cambridge, UK), once in each of the last three weeks of the experiment.

157   In the first of the last three experimental weeks (12 days and 3 days after the long- and short-

158   term drought treatments started, respectively), the driest pots were those of the long-term

159   drought treatment, while the pots of the short-term drought treatment showed an intermediate

160   soil humidity, and the pots of the control treatment showed the greatest humidity (Fig. S1). The

161   soil of the control treatment showed constant humidity during the last three experimental weeks,

162   whereas the pots of both drought treatments showed significant and progressively decreasing

163   humidity across measuring times (Fig. S1).

164   In addition, since *P. rhoeas* grows in presence of no, few or many competitors (e.g.,

165   together with other forbs and grasses; McNaughton & Harper, 1964), we assessed whether the

166   response of plants to the drought treatments differed when they grew or did not grow under

167   competition. In half of the pots of each treatment, we used *Galium album* (Rubiaceae) as

168   competitive species, since *Galium* species are commonly co-occurring with *P. rhoeas*

169   (McNaughton & Harper, 1964). To do so, four seedlings of *G. album* were planted in each

170   corner of the competition pots on the same day as the seedlings of *P. rhoeas* (Fig. 1).

171   **Plant measurements**

172   The wilting time, defined as the day when the leaves of *P. rhoeas* hung over the edge of the

173   pots, was checked daily from the start of the drought treatment. When the plants started to wilt,

174   we harvested them, and the non-wilted plants were harvested at the end of the experiment. After

175   harvesting, the total number of leaves (i.e., the wilted and non-wilted ones in the wilted plants)

176   was counted and the length of the longest leaf was measured to the nearest 0.5 cm. Additionally,

177   roots were carefully dug out and washed to remove the substrate. The length of the longest root

was measured to the nearest 1 mm as an estimate of maximum rooting depth. Plants were then dried in an oven for 72 hours at 60°C and both dry aboveground and belowground biomass (g) were determined. Lastly, the root:shoot ratio was calculated (dry aboveground biomass/dry belowground biomass).

Statistical analysis

All statistical analyses were conducted with R *version* 4.1.1 (R Development Core Team, 2021). Linear Mixed-effect Models (LMM) were applied for each of the seven response variables, using the "lme4" package and the "lmer" function (Bates et al., 2015). Past precipitation predictability treatment (more *versus* less), drought treatment (control, short-term and long-term drought), and competition treatment (without *versus* with competition) as well as their two- and three-way interactions were included as fixed factors. Seed family nested in plot was included as random factor and initial number of leaves as covariate. In all models, we tested the assumptions of normality and homogeneity of variance of the residuals using the Shapiro-Wilk test and the Bartlett test, respectively. If the residuals were not normally distributed, we transformed the response variable (*see* transformations in Table 1). When we found significant interactions, we applied Tukey's post-hoc contrasts using the "lsmeans" package (Lenth, 2016) to understand which levels were significantly different.

Results

Treatment effects on wilting time

Past precipitation predictability led to differences in wilting time in plants exposed to the long-term drought treatment without competition, but neither in the plants with competition, nor in plants exposed to the short-term drought treatment. This result is shown by a significant past

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201   predictability × drought × competition three-way interaction (Table 1, Figs. 2, 3) and the post-

202   hoc contrasts shown in Figure 3. Plants whose progenitors experienced less (past L) predictable

203   precipitation wilted significantly later than those whose progenitors experienced more (past M)

204   predictable precipitation (post-hoc contrast: $p = 0.005$) (Fig. 3). This effect was evident from

205   day 20 to the end of the experiment, when the proportion of wilted plants was higher (i.e., the

206   proportion of non-wilted plants was lower, Fig. 2) if past predictability was M compared to L.

207   Past predictability did not promote significant differences in wilting time under short-term

208   drought or control treatments (Fig. 3). Overall, in presences of droughts, plants without

209   competition wilted significantly later than plants under competition (Figs. 2, 3). None of the

210   plants of the control treatment wilted during the experiment (Figs. 2, 3).

211   **Treatment effects on aboveground traits**

212   Past precipitation predictability led to differences in all three aboveground morphological traits,

213   either in two-way interactions with the drought treatment for aboveground biomass and number

214   of leaves, or in three-way interactions with the drought treatment and competition for maximum

215   leaf length and number of leaves (Table 1). Under long-term drought and without competition,

216   plants from past L showed significantly lower aboveground biomass ($p = 0.015$), shorter leaves

217   ($p = 0.025$) and fewer leaves ($p = 0.047$) than plants from past M (Fig. 4A–C). However, past

218   predictability did not promote significant trait differences under long-term drought with

219   competition. Nor did past predictability significantly affect aboveground trait responses in the

220   control or short-term drought treatments, irrespective of the competition treatment. In addition,

221   plants from past L showed overall significantly shorter leaves compared to the plants from past

222   M, as indicated by the past predictability main effect (Table 1). Furthermore, under long-term

223   drought, significant differences between competition treatments existed for all aboveground

224   traits in plants from past M but in plants from past L only for aboveground biomass (Fig. 4A–

225 C), indicating that the past predictability treatments let to differences in plasticity to

226 competition.

227 **Treatment effects on belowground traits**

228 Past precipitation predictability led to differences in all three belowground traits either in two-

229 way interactions with the drought treatment for belowground biomass (marginally significant,

230 Table 1) and with competition for root length as well as in three-way interactions with drought

231 and competition for belowground biomass and root:shoot ratio (Table 1). Under long-term

232 drought and without competition, plants from past L showed a significantly lower belowground

233 biomass ($p = 0.028$) and a lower root:shoot ratio ($p = 0.036$) than plants from past M (Fig. 4D,

234 F). This effect was also found under control conditions with competition in the root:shoot ratio

235 ($p = 0.043$) but no significant differences were found in the short-term drought treatment (Fig.

236 4F). Under long-term drought, significant differences between competition treatments existed

237 for root:shoot ratio in plants from past L but not in plants from past M, and the opposite was

238 true in the control treatment (Fig. 4F). The effect of past predictability on the root length varied

239 with the competition treatment (Table 1). Across the three drought treatments, plants from past

240 L growing under competition showed significantly shorter roots compared to the plants from

241 past M growing under competition ($p = 0.008$) but no significant differences between the past

242 predictability treatments were found within drought-treatments, independent of whether plants

243 grew with or without competition (Fig. 4E).

244

245 **Discussion**

246 Our study shows that lower precipitation predictability experimentally applied over multiple

247 generations caused drought tolerance in *Papaver rhoeas*. Lower past precipitation predictability

caused trait differences of the offspring plants growing in common-garden conditions, suggesting that precipitation predictability triggered differences in selection. The results on plant wilting show that, under severe drought, plants whose progenitors experienced lower precipitation predictability survived longer than those whose progenitors experienced more predictable precipitation. However, plants from past L had lower biomass and less leaves, suggesting that lower predictability can cause evolution towards a drought-tolerance strategy, helping to reduce water loss. In addition, in line with findings in the previous generations exposed to differences in precipitation predictability (e.g., March-Salas, van Kleunen & Fitze, 2021.; March-Salas et al., 2022), under long-term drought and the control treatment the investment into roots was lower in plants from past L compared to M when they were growing without or with competition, respectively. This also indicates that the root trait expression was shaped by differences in precipitation predictability, and it suggests that lower precipitation predictability led to improved drought stress tolerance rather than to a drought-escape or a drought-avoidance strategy.

Is past precipitation predictability promoting different plant phenotypes under common-garden conditions?

In line with our first hypothesis, simulated precipitation predictability during three consecutive generations caused different plant phenotypes under common-garden conditions. All above- and below-ground traits were significantly affected either by past precipitation predictability as main effect or by interactions of past predictability with drought and/or competition (Table 1). This suggests that differences in past precipitation predictability induced differences in the strength of selection, which would be in line with previous results showing that different precipitation predictability induces differences in the selective regime (March-Salas, Fandos & Fitze, 2021; March-Salas, van Kleunen & Fitze, 2021; Yin et al., 2022). Phenotypic differences

272   shown in our common-garden could also be mediated by maternal effects. However, potential

273   maternal effects were controlled in the models by including early size as a covariate, as maternal

274   effects are mainly expressed in plant early stages (Donohue, 2009). Moreover, early size was

275   not significantly different between past predictability treatments (initial number of leaves: Chi-

276   sq. $= 1.223$; $p = 0.269$; initial largest diameter: Chi-sq. $= 0.858$; $p = 0.354$). Thus, it is unlikely

277   that phenotypic differences found in our common-garden are the result of maternal effects. The

278   observed results are rather the effect of differences in selection, and ultimately, in line with an

279   evolutionary change driven by precipitation predictability.

280   Past precipitation predictability also played a role in the plasticity expressed by the

281   offspring grown under different treatments in the common-garden experiment. Reduced

282   plasticity to competition was found in plants from past L compared to plants from past M in

283   above- and below-ground biomass, leaf length, and number of leaves if they were exposed to

284   the long-term drought treatment (Fig.4). This result is consistent with other studies suggesting

285   that the precipitation predictability in the environment of origin can shape the evolution of

286   phenotypic plasticity (Yin et al., 2022) or that the precipitation predictability of the maternal

287   environment determines the variation in the descendants' responses (March-Salas et al., 2022;

288   Leung et al., 2022). Since environments that fluctuate less predictably induce mismatches

289   between plastic responses and selective pressures, organisms may evolve lower plasticity under

290   decreased predictability (Leung et al., 2020). However, the degree and direction of plasticity to

291   competition differed between past predictability treatments and drought treatments in

292   root:shoot ratio (Fig. 4F). These findings thus indicate that the effect of precipitation

293   predictability on the direction of plastic responses depended on the measured trait, and that

294   differences in plasticity to competition were mainly expressed under the long-drought

295   treatment.

296   Strong plasticity in adaptive traits can hinder rapid evolution (Metz et al. 2020). In our

297   previous experiments on *P. rhoeas*, progenitor plants exhibited reduced transgenerational

298   plasticity in root functional traits and investment strategies under less predictable conditions

299   compared to those under more predictable conditions (March-Salas et al., 2022). In our

300   common-garden experiment, plants from lower past precipitation predictability showed smaller

301   plant size and lower root investment in the presence of long-term drought. Our results thus

302   suggest that reduced plasticity caused by lower predictability goes hand-in-hand with rapid

303   evolution of traits while evolutionary shifts are less likely if progenitors showed high trait

304   plasticity, in line with our second hypothesis and other studies (Merilä & Hendry, 2014; Metz

305   et al. 2022).

306

307   **Is past precipitation predictability affecting the strategy expressed by offspring**

308   **plants under drought stress?**

309   In line with our third hypothesis, our results indicate that the degree of precipitation

310   predictability experienced during three consecutive generations caused differences in the

311   plants' responses to severe drought events. Overall, in the long-term drought treatment, plants

312   from past L started wilting significantly later than those from past M, which shows longer plant

313   survival if progenitor plants were exposed to lower precipitation predictability. Higher survival

314   rates under less predictable conditions were also found in the experiments with the progenitors

315   (March-Salas & Fitze, 2019a), showing the relevant role of precipitation predictability in

natural selection processes. Lower predictability entails less-regular water availability, what may pre-adapt plants to certain drought events and to environmental variation.

The presumed longer survival of plants from past L may be explained by their reduced size. Plants from past L showed lower biomass, smaller leaves and a lower number of leaves. These phenotypic responses may help to better withstand water-deficit conditions (Lei et al., 2006), since smaller size prevents plants from water loss through transpiration (Aroca, 2012; Chaves, 2002). This can be understood as a physiological adjustment induced by the progenitor's precipitation predictability (March-Salas & Fitze, 2019b) and allows plants to tolerate drought stress (Májeková et al., 2019) and increase their leaf water content (Blum, 2005; Bartlett et al., 2014). This indicates a drought-tolerance strategy promoted by reduced growth and likely driven by rapid evolution of plants in response to lower precipitation predictability preparing them as well against drought events (Koziol et al., 2012; Rauschkolb et al., 2022). Past precipitation predictability thus resulted in changes in the morphological and physiological trait expression that may help plants to avoid future extinction of populations under climate change (Maggio et al., 2018).

Developing a deeper root system or greater root biomass can help plants to avoid drought, since obtaining water from deeper soil layers prevents water deficit and increases survival rates (Aroca, 2012; Gowda et al., 2011; Kavar et al., 2008; Padilla & Pugnaire, 2007). Across all past predictability and competition treatments, our experiment shows a lower root biomass and shorter roots but a greater relative investment into roots (i.e., the root:shoot ratio) in plants under longer drought (compared to the control and short-term drought treatment). However, the root response to drought depended on the past predictability precipitation. Under long drought conditions and no competition, plants from past L produced lower root biomass and lower root:shoot ratio than plants from past M. This shows that the progenitor's

precipitation predictability regime leads to a different root response under severe drought of its offspring, suggesting an adaptive response to lower predictability in water availability (March-Salas et al., 2022; Yamauchi et al., 2021; Zhou et al., 2019). Plants of the control and competition treatment also showed a greater root response in plants from past M compared to those from past L, which could be related to a water deficit mediated by the competitor plants. In the progenitor generations, plants under L exhibited reduced root investment, which increased their fitness (March-Salas et al., 2022). Lower predictability more likely reduces the length of the primary root while it can promote lateral root branching in order to help plants to withstand stressful conditions such as water deficits (Gallego-Tévar et al., 2022; March-Salas et al., 2022). This strategy is expected in annual species, which aim to absorb available resources close to the soil surface, but not necessarily in perennial plants, which generally root deeper (March-Salas, van Kleunen & Fitze, 2021). Overall, the root trait responses show that lower precipitation predictability is unlikely to induce a drought-avoidance strategy and rather induce a functional stress tolerance mechanism. Nevertheless, a drought-escape strategy cannot be discarded as possible outcome in this study, because experimental plants did not flower before wilting and thus it was not possible to test this hypothesis.

In addition, for all traits except root:shoot ratio, no effects of past precipitation predictability existed under competition. In the short and long drought treatments, competition for limiting resources led to no morphological changes between plants with different past precipitation predictability, as would be expected (Craine & Dybzinski, 2013; Johansson, 2008). Given that no significant differences in seedling size existed between past precipitation predictability treatment, the observed differences without competition occurred thereafter through differential growth and strategy, i.e., when the drought treatments already started to show effects. Competition thus accelerated death and reduced plant size, but it also hindered

the development of phenotypic differences between plants with different past precipitation predictability. This result is most likely because water availability was even lower in pots with competition, as more plants competed for the limiting resources in the drought treatments, preventing differential functional strategies. Our results are in line with previous theoretical studies indicating that interspecific competition may influence the evolutionary trajectories of plant populations (Craine & Dybzinski, 2013; Johansson, 2008). This then indicates that potential evolutionary changes driven by precipitation predictability may not manifest if plants are exposed to competition.

Conclusion

An organism's evolutionary response to differences in environmental predictability is of great interest given the current and projected reduction in climatic predictability. Here, we assessed whether past precipitation predictability promoted a specific functional strategy to their offspring to deal with longer drought episodes. The results of this study show, to our knowledge for the first time, that ancestors exposed to lower precipitation predictability produced offspring with increased drought tolerance, as they took longer to wilt when exposed to a long drought episodes. This supports earlier results on the progenitor generations (i.e., March-Salas et al., 2019, 2022; March-Salas & Fitze, 2019a), which showed that less predictable precipitation may not be detrimental for plants and may rather favour rapid evolutionary responses. Lower predictability may cause more prolonged droughts, which is likely to be a key factor driving the observed evolutionary changes. However, phenotypic differences disappeared if plants were grown under competition, indicating that interspecific competition may slow down evolutionary trajectories driven by precipitation predictability. In conclusion, our study

387 provides new insights into the evolution of plants in a changing climate, highlighting that

388 precipitation predictability is an important evolutionary driver of plant drought strategies.

Conflict of interest

All authors declare there are no conflicts of interest.

Author contributions

MMS with PSF conducted the evolutionary experiments that gave rise to the original seed material. KS, TC, JFS and MMS designed the common-garden experiment. KS and TC conducted the experiment, measured the plants and analysed the data with inputs from JFS, VH and MMS. KS, TC and MMS wrote the first draft of the manuscript, and all authors contributed to revisions.

Data availability statement

Data will be made available in a public repository upon acceptance for publication.

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Figure legends

Figure 1: Design of the common-garden experiment. Each square represents one pot for each treatment level. Drought treatments are indicated by different background colours (grey: control treatment, yellow: short-term drought treatment, orange: long-term drought treatment). Blue and red dots represent *Papaver rhoeas* individuals whose progenitors experienced more (M) or less (L) predictable precipitation, respectively. In pots with competition, four individuals of *Galium album* (green dots) were planted in each corner. For each treatment level, we used six seed families with four replicates, resulting in 288 pots.

Figure 2: Proportion of non-wilted *Papaver rhoeas* plants during the experiment per treatment level. The grey lines represent the control treatment, yellow the short-term drought treatment, and orange the long-term drought treatment. Within each drought treatment level, thin lines represent plants whose progenitors experienced more (M) predictable precipitation while thick lines represent those that experienced less (L) predictable precipitation. Solid lines show *P. rhoeas* plants without competition while the dashed lines show plants growing with competition. The x-axis shows the number of days since the start of the long-term drought treatment.

Figure 3: Number of days until *Papaver rhoeas* plants started to wilt per treatment combination. The panels show the different drought treatments, while the past precipitation predictability and competition treatments are represented on the x-axis. Blue and red colours indicate the plants whose progenitors were exposed to more or to less predictable precipitation, respectively. Note that no plants of the control group wilted during the experiment. Mean ± standard error (SE) are shown in the bar plots. Post-hoc contrasts among all treatment combinations are indicated with letters. Sample size per treatment combination is indicated at the bottom of each bar.
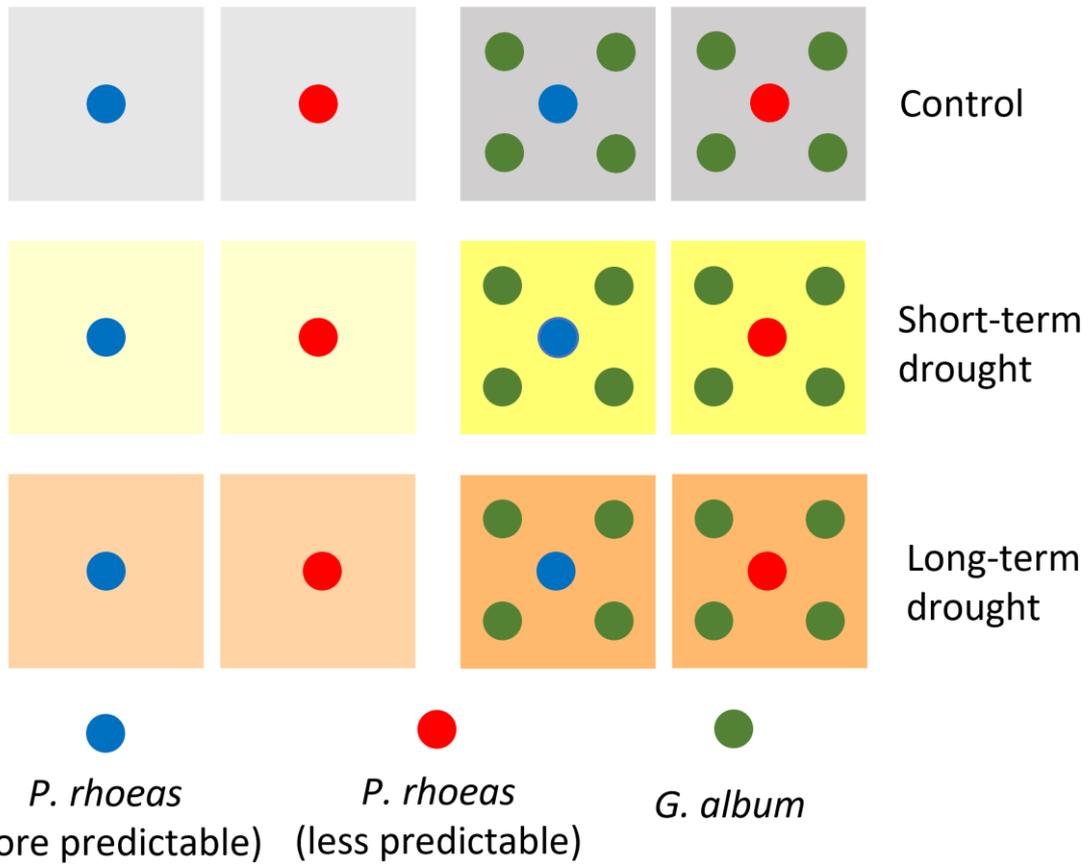
Figure 4: Treatment effects on morphological traits of *Papaver rhoeas*: (A) Dry aboveground biomass; (B) Length of the longest leaf; (C) Number of leaves; (D) Dry belowground biomass; (E) Root length; and (F) Root:shoot ratio. The panels show the different drought treatments, while the past predictability and competition treatments are represented on the x-axis. Blue and red colours indicate the plants whose progenitors were exposed to more or to less predictable precipitation, respectively. Mean ± standard error (SE) are shown. Post-hoc contrasts among all treatment combinations are indicated with letters.

588   **Table 1:** Results of the linear mixed-effects model including the effect of past precipitation predictability [P], competition [C], drought [D] and their

589   two- and three-way interactions on trait responses of *Papaver rhoeas*. Initial number of leaves was also included as covariate. Shown are Chi-square

590   values followed by significance stars: * $0.05 > p \geq 0.01$; ** $0.01 > p \geq 0.001$; *** $p < 0.001$, and • reflects marginal effects ($0.1 > p \geq 0.05$). Sample

591   size was 284. *df*, degrees of freedom. Response variables are wilting time (defined as number of days until wilting), aboveground biomass (g),

592   belowground biomass (mg), root:shoot ratio, root length (cm), number of leaves, and length of the longest leaf (cm).
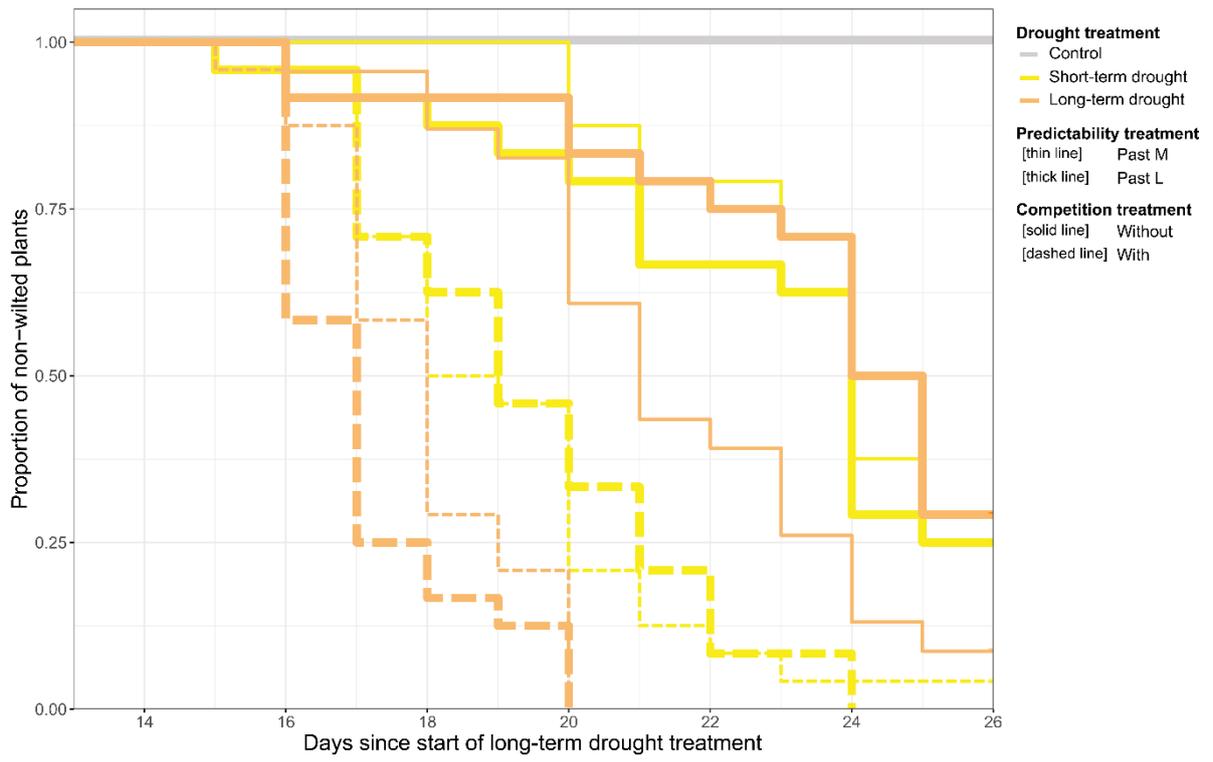
Effect	df	Wilting time[1]	Aboveground biomass[2]	Length of longest leaf[3]	Number of leaves[4]	Belowground biomass[5]	Root length[5]	Root:shoot ratio[6]
Initial number of leaves	1	**21.281 *****	**25.730 *****	**9.489 ****	**44.120 *****	**24.198 *****	0.807	0.712
Precipitation predictability [P]	1	0.012	1.881	**4.276 ***	0.074	0.581	1.186	0.030
Competition [C]	1	**186.271 *****	**381.041*** **	**79.409 *****	**182.009 *****	**339.743 *****	**153.125 *****	0.465
Drought treatment [D]	2	**672.698 *****	**156.845 *****	**153.852 *****	**66.624 *****	**69.765 *****	**23.125 *****	**31.891 *****
P x C	1	1.382	0.269	0.871	3.800 •	0.007	**7.872 ****	0.088
P x D	2	**7.201 ***	**6.162 ***	3.533	**7.037 ***	4.729 •	0.769	2.875
C x D	2	**91.665 *****	**17.215 *****	**51.595 *****	**13.261 ****	**7.620 ***	1.981	**11.293 ****
P x C x D	2	**18.269 *****	3.500	**8.176 ***	**7.954 ***	**12.683 ****	4.726 •	**24.701 *****

Transformations: [1] ^3.5; [2] ^0.55; [3] ^0.8; [4] ^0.12; [5] ^0.42; [6] ^0.7

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		Control
		Short-term drought
		Long-term drought

P. rhoeas
(more predictable)

P. rhoeas
(less predictable)

G. album
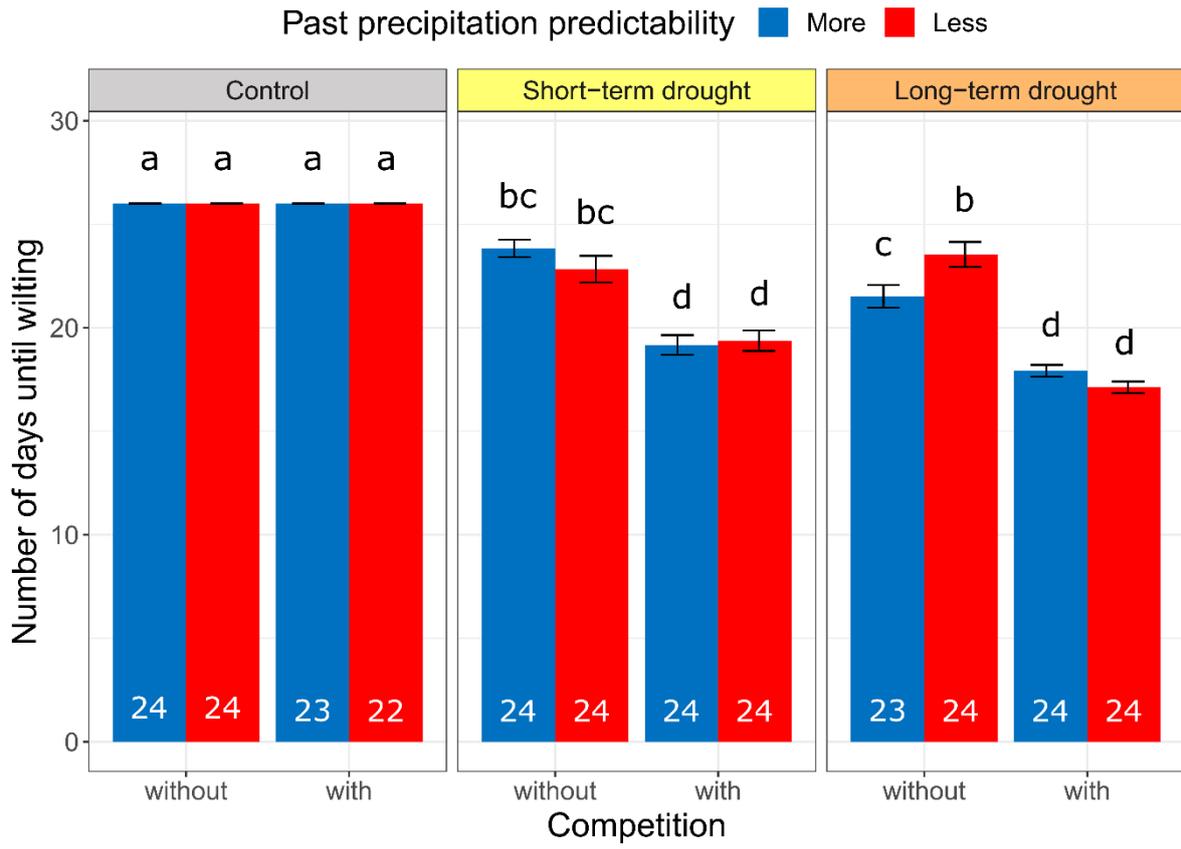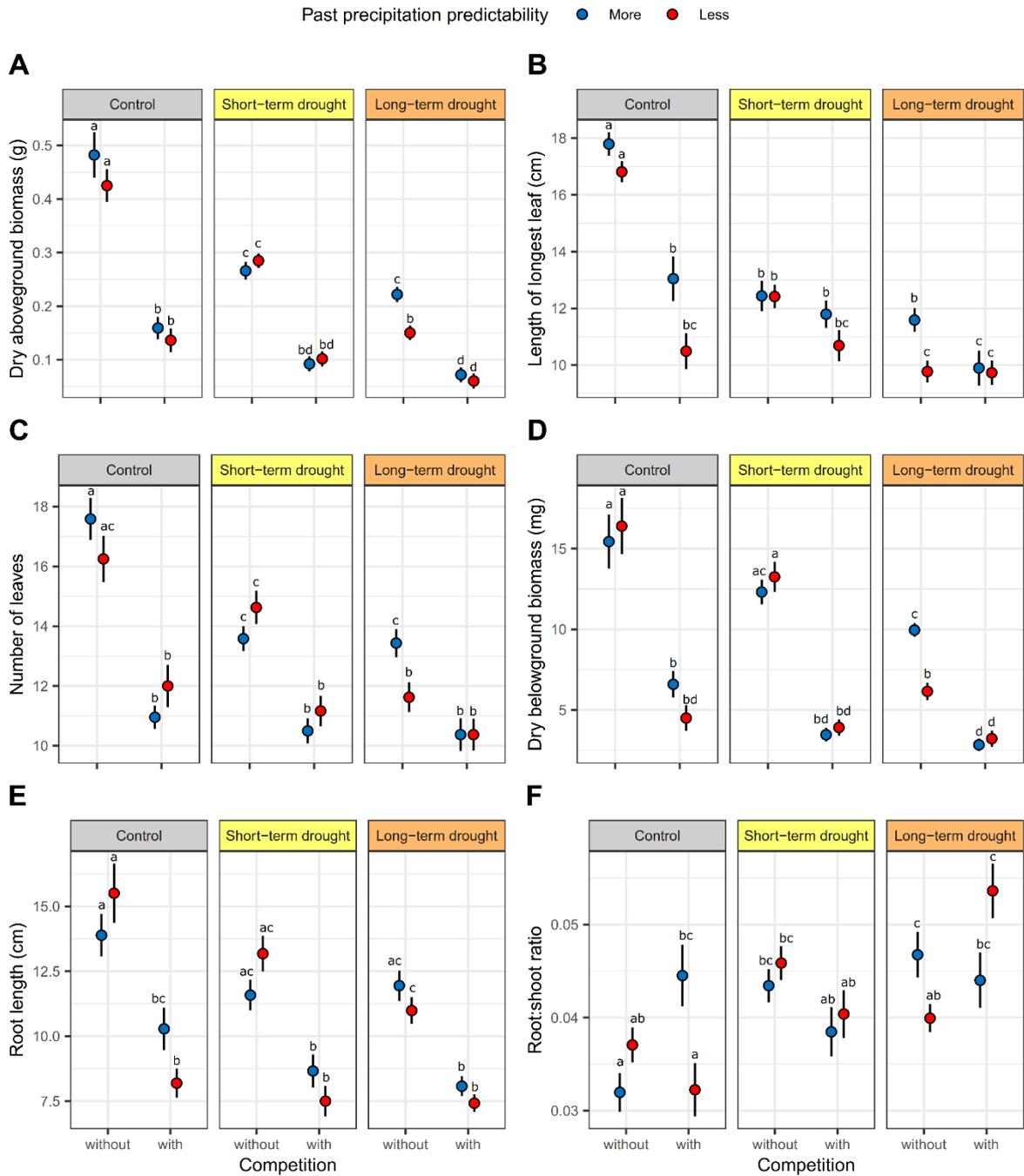
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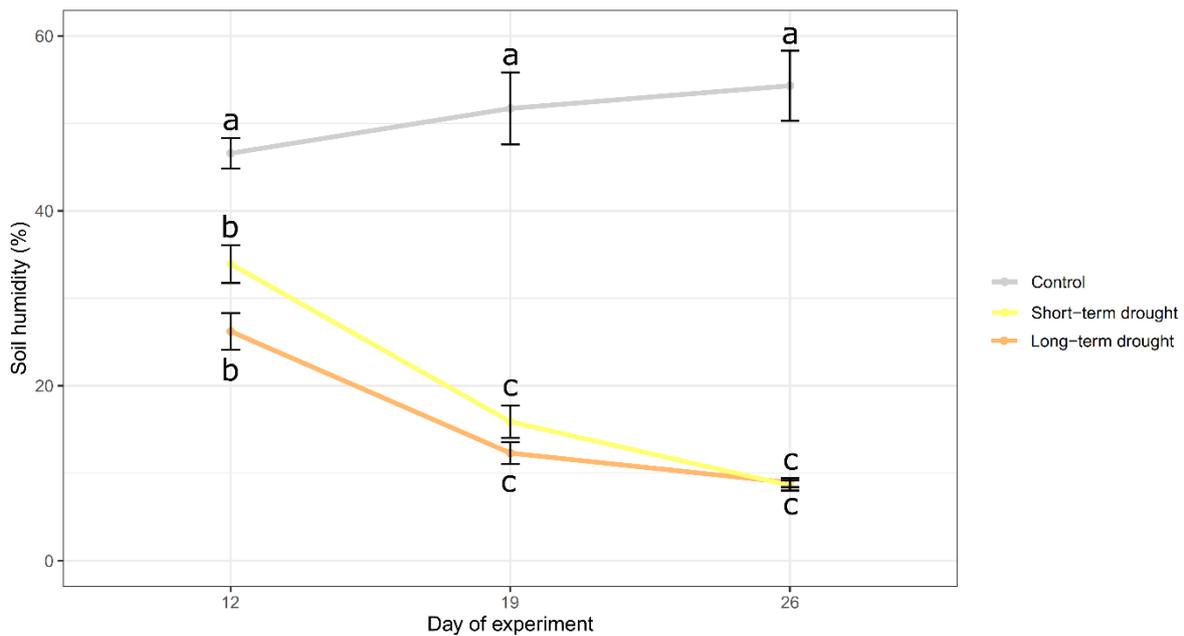**Figure 1**

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598 **Figure 2**

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Figure 3

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Figure 4

603 **Supplementary Material:**

604 **Supplementary data legends**

605 **Figure S1:** Relative soil humidity (%) measured in the pots per drought treatment and time.

606 Relative soil humidity was measured in each of the last three weeks of the experiment (i.e., 12,

607 19 and 26 days after the long drought treatment started). The relative soil humidity in the control

608 treatment is coloured in grey, the short-term drought treatment in yellow, and the long-term

609 drought treatment in orange. Significant post-hoc contrasts among time-measures within each

610 drought treatment level are indicated with letters. The mean temperature in the greenhouse was

611 20.1 °C and the mean light intensity was 2,300 lux.

612