

Rapid and positive responses of plants to lower precipitation predictability

Martí March-Salas^{1,2}, Mark van Kleunen^{3,4} and Patrick S. Fitze^{1,2}

¹Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), José Gutiérrez Abascal 2, 28006 Madrid, Spain

²Department of Biodiversity and Ecologic Restoration, Instituto Pirenaico de Ecología (IPE-CSIC), Avda. Nuestra Señora de la Victoria 16, 22700 Jaca, Spain

³Department of Biology, University of Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany

⁴Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, People's Republic of China

 PSF, 0000-0002-6298-2471

Current climate change is characterized by an increase in weather variability, which includes altered means, variance and predictability of weather parameters, and which may affect an organism's ecology and evolution. Few studies have experimentally manipulated the variability of weather parameters, and very little is known about the effects of changes in the intrinsic predictability of weather parameters on living organisms. Here, we experimentally tested the effects of differences in intrinsic precipitation-predictability on two herbaceous plants (*Onobrychis viciifolia* and *Papaver rhoeas*). Lower precipitation-predictability led to phenological advance and to an increase in reproductive success, and population growth. Both species exhibited rapid transgenerational responses in phenology and fitness-related traits across four generations that mitigated most effects of precipitation-predictability on fitness proxies of ancestors. Transgenerational responses appeared to be the result of changes in phenotypic plasticity rather than local adaptation. They mainly existed with respect to conditions prevailing during early, but not during late growth, suggesting that responses to differences in predictability during late growth might be more difficult. The results show that lower short-term predictability of precipitation positively affected fitness, rapid transgenerational responses existed and different time scales of predictability (short-term, seasonal and transgenerational predictability) may affect organisms differently. This shows that the time scale of predictability should be considered in evolutionary and ecological theories, and in assessments of the consequences of climate change.

Keywords:

local adaptation, environmental predictability, inter-seasonal predictability, multiple-generation experiment, phenotypic plasticity, transgenerational response

Authors for correspondence:

Martí March-Salas

e-mail: martimarchsalas@gmail.com

Patrick S. Fitze

e-mail: patrick.fitze@mncn.csic.es

1. Introduction

Current global climate change affects averages and variance of environmental conditions and it decreases the temporal predictability of weather events (the degree of the temporal autocorrelation; [1]). Changes in the intrinsic environmental predictability [2] may affect individual life histories and phenotypic expression [3,4]. Theoretical work shows that the effects of differences in environmental predictability may be greater than those of differences in demographic stochasticity, because environmental predictability is predicted to operate at all population sizes with equal strength [4]. Low environmental predictability may negatively affect life-history traits [5] and thereby population growth. Low environmental predictability also favours earlier reproduction [6], but while classic theory states that this should be at the cost of investment in individual offspring [7], more recent theory states that it should increase investment in individual offspring at the cost of the number of offspring [8]. Moreover, the degree of intrinsic environmental predictability might be key to whether and how organisms may adapt to environmental change [9]. Greater predictability favours adaptive changes [10], while lower predictability rather favours changes of phenotypic plasticity [10–12]. However, these theories remain largely untested [13].

Current climate change is characterized by increased variation (increased fluctuations around the average) of different weather parameters [14] and a reduction in the predictability of precipitation [15], which may potentially affect selection acting on many living organisms [16]. Moreover, lower predictability may increase extinction risk and reduce population persistence [4] and it has different temporal dimensions. It can refer to the regularity in the timing and magnitude of environmental fluctuations over a short time scale (e.g. autocorrelation among daily or weekly measures), or it can refer to fluctuations over larger time scales (e.g. autocorrelation among seasonal or annual measures, [17]). The effects of weather predictability and different temporal dimensions are far from being understood, despite their potential importance for organisms, especially in the current era of rapid climate change.

Here we experimentally tested the effects of differences in intrinsic intra- and inter-seasonal predictability on two plant species. For the intrinsic intra-seasonal predictability, we simulated more (M) and less (L) predictable daily precipitation, i.e. differences in the predictability of the summed simulated and natural daily precipitation. In M, the probability and timing of rainfall were more predictable (higher autocorrelation among days), while in L, both were less predictable. For the intrinsic inter-seasonal predictability, we simulated more and less predictable precipitation between seasons (between spring: during early plant growth, and summer: during late plant growth). Thus, plants were exposed to higher inter-seasonal predictability (MM, LL) or lower inter-seasonal (ML, LM) predictability, or in other words, to a higher or lower autocorrelation of precipitation between early and late growth (electronic supplementary material, figure S1). To test whether treatment effects may depend on the year, this experimental design was repeated in four consecutive years.

Widely accepted theory claims that high transgenerational predictability may lead to rapid adaptation [9,12]. This suggests that populations in all experimental predictability regimes (LL, MM, LM, ML) should rapidly respond if subsequent generations are exposed to the same precipitation regime (i.e. if transgenerational predictability is high). We tested this hypothesis by planting in the following three years (2013, 2014 and 2015) descendant seeds (G_1 , G_2 and G_3 , respectively) in plots exposed to the treatment combination experienced by their mother (e.g. seeds of LL mothers were planted in LL plots; electronic supplementary material, figure S1). This design allowed tracking transgenerational responses within matriline over three descendant generations (G_1 – G_3 ; electronic supplementary material, figure S1). To test for local adaptation to simulated intrinsic precipitation-predictability, G_2 - and G_3 -descendant seeds of the four types of matriline (LL, MM, LM, ML) were planted, in the matriline's treatment and also in the other three treatment combinations (in 2014 and 2015, respectively), using a reciprocal transplant experiment [18].

According to theory, we expected that: (i) less predictable precipitation will lead to a phenological advance, (ii) less predictable precipitation will negatively affect reproductive success and population growth; and (iii) both species may exhibit rapid transgenerational responses allowing them to cope with differences in the predictability of precipitation. If transgenerational responses reflect local adaptation to precipitation-predictability, we expected that (iv) descendants of a

given matriline will perform better in the matriline's treatment than descendant of matriline of the other precipitation treatments.

2. Material and methods

(a) Study species and sowing design

Onobrychis viciifolia Scop. (Fabaceae, perennial) and *Papaver rhoeas* L. (Papaveraceae, annual) seeds (electronic supplementary material, study species) were sown in natural environments located at the experimental field station 'El Boalar' (Jaca, Huesca, Spain) and exposed to different intrinsic precipitation-predictability regimes. These seeds originated from geographically close sites, which exhibit higher precipitation than the study site, but otherwise similar climates (electronic supplementary material, seed origin), and they are hereafter referred to as the ancestral generation (G_0). G_0 seeds used for the experiments were randomly chosen and individually sown in early April of four consecutive years (2012–2015) in each of 16 enclosures (electronic supplementary material, figure S2). Enclosures consisted of two planting plots each (one for *P. rhoeas* and one for *O. viciifolia*). Seeds produced by G_0 are hereafter referred to as G_1 , those produced by G_1 as G_2 , and those produced by G_2 as G_3 . G_1 , G_2 and G_3 are thus descendants and they were sown in 2013 (G_1), 2014 (G_2) and 2015 (G_3) in the same treatment combination as their mother (electronic supplementary material, figure S1) to test whether predictability treatments induce differences in the strength and direction of transgenerational responses. While descendants were potentially able to exhibit a transgenerational response, by experimental design, ancestors were unable to do a transgenerational response with respect to the experimental conditions. Thus, differences among ancestors planted in different years represent differences owing to variation among years, while differences between ancestors and descendants growing in the same plot and year represent transgenerational responses. To test for local adaptation to the matriline's precipitation-predictability treatment, a reciprocal transplant experiment was conducted [18], and G_2 - and G_3 -descendants of the four types of matriline were sown in 2014 and 2015 in all four treatment combinations (LL, LM, ML, MM). To make sure that treatment-induced transgenerational responses would not be confounded with transgenerational responses to plot specific conditions, seeds were never planted in the plot in which the mothers had been growing previously. To increase the chance that one seedling emerged per planting position, multiple seeds were sown per planting position. In the case that several seedlings emerged in the same planting position, all but one of the seedlings was thinned to avoid competition. There were no significant differences between thinned and non-thinned seedlings (all $p \geq 0.8$; electronic supplementary material, experimental system, sowing and thinning protocol). To test whether initial seed mass predicts reproductive success, each seed of *O. viciifolia* was individually weighed previous to sowing, and in *P. rhoeas*, 10 randomly chosen seeds produced by the same mother were weighed to determine the average seed mass.

(b) Precipitation-predictability treatment

For each species, eight experimental outdoor plots were subjected to more (M) and another eight plots to less (L) predictable precipitation. To simulate differences in intrinsic intra-seasonal precipitation-predictability, the field site's (natural) precipitation-predictability was manipulated by providing M- and L-plots with supplemental precipitation at regular and random intervals, respectively, using an automatic sprinkler system. M-plots were irrigated 14 times per week, each irrigation event lasted 5 min and irrigation happened at constant time-intervals, i.e. for 10 min on each day. L-plots were also irrigated 14 times

per week, each irrigation event lasted 5 min, and irrigation happened at randomly chosen time points. Each plot and treatment obtained the same number of precipitation events and the same amount of total (natural + supplemental) precipitation per week. Irrigation happened from sowing to the end of the plant's annual life cycle (for approx. 4.25 months) and each irrigation event provided 1.3 mm m^{-2} of supplemental precipitation, which sums up to approximately 330.2 mm of additional supplemental precipitation per year, and together with the natural precipitation it corresponds to the natural precipitation conditions from where the seeds stemmed from (electronic supplementary material, text). During 2013–2015, precipitation falling at the field site exhibited no seasonality (electronic supplementary material, figure S3a), the average annual precipitation at the field site was 916 mm (2012–2015), and maximum annual precipitation registered at the field site in the last 10 years was 1265 mm m^{-2} . The intrinsic predictability of the total precipitation (natural + supplemental) estimated by permutation entropy [2] was 0.77 in the M-plots, which was equal to the predictability of the natural precipitation of the study site. In L-plots, it was 0.86, and thus, 11.2% lower than that of the M-plots [19]. Supplemental precipitation was provided during the entire annual life cycle of the plants (4.25 months), which can last in some natural populations until September [20]. This design allowed for an assessment of differences in the intrinsic precipitation-predictability on different above-ground components (including late life-stages), and it avoided the drying up of the plants owing to rapidly raising ambient temperatures (high evapotranspiration). At the field site, average monthly temperatures rise from February to July/August (maximum average monthly temperature was $19.74^\circ\text{C} \pm 0.5 \text{ s.e.}$ in August—mean of the four experimental years; electronic supplementary material, figure S3b), and drying up can occur from June to August when the average monthly temperatures are greater than 16°C , i.e. before the plants complete their annual life cycle. The experimental design thus represents a conservative measure of the effects of intrinsic precipitation-predictability, as the experimental plants were able to complete their life cycle. Moreover, in each plot, the total precipitation (natural precipitation + supplemental precipitation) was within the natural limits registered historically at the study site. The minimum daily precipitation at the study site was identical to the minimum total precipitation in the plots, and the maximum total precipitation did not significantly exceed the natural precipitation or variance observed at the study site during the growing season ($F_{1,62} \leq 0.0003$, $p \geq 0.98$).

To simulate differences in intrinsic inter-seasonal predictability, half of the plots of each early treatment (spring) were either changed to the other treatment (ML, LM) or the same treatment was maintained (MM, LL) during late growth (summer), i.e. from the end of June/beginning of July until end of October. Thus, MM and LL plots were exposed to more, and ML and LM plots to less predictable inter-seasonal precipitation (electronic supplementary material, figure S1, precipitation regime).

(c) Data collection

Emergence of seedlings was checked daily during the first four weeks to determine the probability that a seedling emerged and to determine the time (number of days) passed from sowing to seedling emergence. The time to flowering corresponds to the number of weeks passed from seedling emergence to the appearance of the flower bud in *P. rhoeas* and to the opening of the first flower in *O. viciifolia*. Once fruits were ripe, seeds of each fruit and plant were stored in separate paper bags under cold and dry conditions. Once all seeds of a plant had been collected, the plant was harvested by carefully digging it out. Plants that did not produce seeds were harvested at the end of the growing season (i.e. annual cycle). Of all reproductive plants, individual

seed mass (in mg for *P. rhoeas* and in g for *O. viciifolia*) and the number of produced seeds were recorded.

(d) Statistical analysis

Statistical analyses were conducted for each species separately, using generalized linear mixed-effect models (GLMMs) and the *lme4* package in R (version 3.3.1; [21]). All models contained the fixed factors: early treatment (levels: more versus less predictable precipitation), late treatment (more versus less predictable precipitation) and year (2012, 2013, 2014, 2015), their interactions and plot as a random factor.

The probability of seed production was defined as the probability that a planted seed germinated (i.e. the probability that at least one planted seed per planting position germinated; see the electronic supplementary material, text), survived and reproduced. Per capita population growth rate was calculated following the Ricker's (r) equation: $r = \ln(N_t/N_{t-1})$ [22]. One seedling per planting position was allowed to grow and N_{t-1} thus corresponds to the number of positions per plot (electronic supplementary material, figure S2), while N_t corresponds to the number of produced seeds per plot.

Treatment effects on fitness-related traits of ancestors were tested in four years (2012–2015) and transgenerational responses in descendants (G_1 , G_2 , G_3) were tested in 2013, 2014 and 2015, by comparing descendants and ancestors (G_0) grown in the same plot. GLMMs on transgenerational responses additionally included a fixed factor: potential transgenerational response (PTR, with two levels: ancestors and descendants), all possible interactions with PTR and the random factor matriline identity (ID) (ID of the G_0 mother). In these analyses, a significant PTR \times treatment interaction shows that ancestors and descendants respond differently to the precipitation treatments, which points to a transgenerational response. A significant treatment \times year \times PTR interaction indicates that the treatment-induced transgenerational response differs among generations.

Analyses of the reciprocal transplant experiment additionally included descendant treatment (the treatment to which a descendant seed was exposed) and matriline treatment as fixed factors, their interactions and plot and matriline ID as random factors. The power to detect a significant interaction between descendant treatment \times matriline treatment in G_2 - and G_3 -descendants of the magnitude of the late treatment effect observed in ancestors (figure 1e,f) was calculated using G*POWER 3.1.9.2 [23].

Models with binomial error distribution were used for the probability of seed production, and for all other variables, models with Gaussian error distributions were used. For all statistical analyses, the most parsimonious model was determined using stepwise backward elimination, and all significant parameters in the full models were confirmed in the reduced models, and vice versa. Post hoc tests (*lsmeans* package) were applied using Tukey's HSD test, whenever significant factors (main effects or included in interactions) consisted of more than two levels. For all statistical models, the underlying assumptions were tested, and in the cases where the normality assumption was not met, the response variable was transformed (electronic supplementary material, tables S1, S3 and S4). In the presence of heteroscedasticity and if transformation did not resolve the problem, weighted least square regression was applied. There was no evidence for overdispersion and zero-inflation in any of the binomial models.

3. Results

(a) Effect of precipitation-predictability on phenology, reproduction and population growth

In the more predictable treatment (M), seedlings of ancestors of *P. rhoeas* emerged $19.94 \pm 0.56 \text{ s.e.}$ days after sowing, and

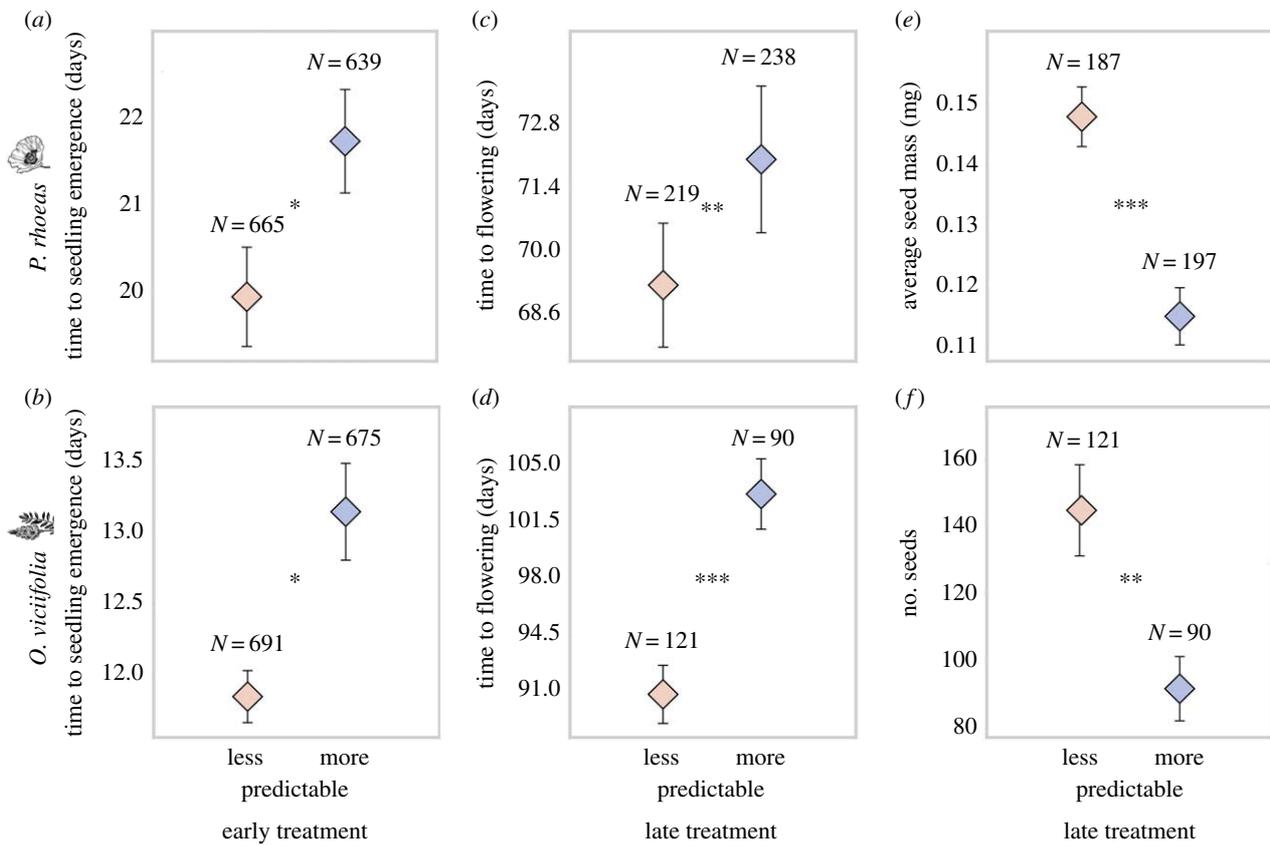


Figure 1. Treatment effects on phenology and fitness-related traits of ancestors of *P. rhoeas* and *O. viciifolia*. Early treatment effect on time to seedling emergence of *P. rhoeas* (a) and *O. viciifolia* (b). Late treatment effect on time to flowering of *P. rhoeas* (c) and *O. viciifolia* (d). Effect of late treatment on fitness-related traits: average seed mass of *P. rhoeas* (e) and number of seeds produced by *O. viciifolia*. (f) Significant differences between less and more predictable precipitation are indicated with asterisks (* $0.05 > p \geq 0.01$; ** $0.01 > p \geq 0.001$; *** $p < 0.001$). Means \pm s.e. of absolute measures are shown and above the estimates sample size (N) per treatment level.

in the less predictable treatment (L), 21.71 ± 0.59 s.e. days after sowing (figure 1a). In M, *O. viciifolia* seedlings emerged 11.85 ± 0.18 s.e. days after sowing, and in L, 13.14 ± 0.34 s.e. days after sowing (figure 1b). Thus, *P. rhoeas* and *O. viciifolia* emerged 1.8 and 1.3 days earlier in the less predictable treatment, showing that significant (electronic supplementary material, table S1) but small treatment effects on emergence time existed in both species. In M of the late treatment, flowering started on average 72.03 ± 1.61 s.e. and 103.18 ± 2.17 s.e. days after emergence in *P. rhoeas* and *O. viciifolia*, while in L of the late treatment, flowering started 69.23 ± 1.33 s.e. and 90.79 ± 1.82 s.e. days after emergence. In *P. rhoeas*, flowering thus started on average 2.8 days earlier in the less predictable treatment (figure 1c), while in *O. viciifolia*, it started 12.4 days earlier (figure 1d). In both species, these differences were significant (electronic supplementary material, table S1), showing that treatment affected the phenology of both species.

Fitness-related traits were significantly and positively affected by L (electronic supplementary material, table S1; figure 1e,f). First, in both species, earlier flowering in L was associated with a higher probability of seed production (electronic supplementary material, table S1: $P_{\text{seed production}}$). While *O. viciifolia* exposed to L during early and late growth increased the probability of seed production in all 4 years (i.e. there were no significant interactions of treatments with year), in *P. rhoeas*, treatment effects depended on the year (electronic supplementary material, table S1: significant early \times year and late \times year interactions), suggesting that inter-annual

differences affect *P. rhoeas* more than *O. viciifolia*. Second, treatment did not affect the number of produced seeds in *P. rhoeas* (treatments effect: $\chi^2_1 \leq 1.147$, $p \geq 0.3$, electronic supplementary material, table S2), and *O. viciifolia* produced significantly more seeds when exposed to L during late growth (electronic supplementary material, table S1; figure 1f). Third, in *P. rhoeas*, L positively affected per offspring investment (electronic supplementary material, table S1: average seed mass; figure 1e), whereas in *O. viciifolia* per offspring investment depended on the year-specific effects of early and late treatment (significant three-way interaction; electronic supplementary material, table S1).

To assess the relevance of individual seed mass for both plant species, we tested its effect on different multiplicative fitness components (electronic supplementary material, table S3). In *O. viciifolia*, the probabilities of seedling emergence and seed production, and the number and mass of produced seeds were not significantly affected by initial seed mass (all $p \geq 0.5$, electronic supplementary material, table S3), showing that in *O. viciifolia*, treatment-induced differences in seed mass and inter-annual variation in seed mass (electronic supplementary material, table S1) do not affect reproductive success. In *P. rhoeas*, initial seed mass positively affected the number of produced seeds ($p = 0.006$), but not the probabilities of seedling emergence and reproduction, or the average mass of the produced seeds (all $p \geq 0.1$, electronic supplementary material, table S3). Moreover, in both species, the number of produced seeds was not significantly affected by interactions between treatments and the covariates appearing in

electronic supplementary material, table S3 (all $p > 0.1$), which suggests that the strength of selection acting on these traits was similar in all four treatment combinations.

In *O. viciifolia*, the per capita population growth rate in L of the early and late treatment was 38% and 28% higher than in M (early treatment: $\chi_1^2 = 12.57$, $p < 0.001$; late treatment: $\chi_1^2 = 7.29$, $p = 0.007$). Similarly, in *P. rhoeas*, per capita population growth rate was 28% higher in L of the early treatment ($\chi_1^2 = 4.18$, $p = 0.041$), and it was not affected by the late treatment ($\chi_1^2 = 0.90$, $p = 0.342$).

(b) Transgenerational responses

PTR was significant in most traits and in both species (figure 2; electronic supplementary material, table S4). For example, in *P. rhoeas*, the probability of seed production was affected by a significant year \times PTR (ancestors versus descendants) interaction (figure 2a; electronic supplementary material, table S4). No significant differences existed between G_1 -descendants and ancestors (in 2013), G_2 -descendants were more likely to produce seeds than ancestors (in 2014) and G_3 -descendants were significantly more likely to produce seeds than ancestors (in 2015). Over only three generations, the transgenerational response exhibited by *P. rhoeas* increased the probability that a sown seed would result in a successfully reproducing plant by 16% (figure 2a).

Interactions between treatments and PTR show that transgenerational responses existed with respect to precipitation-predictability. Significant interactions existed in the probability of seed production (figure 2b) and the number of produced seeds (figure 2f) in *O. viciifolia*, in average seed mass (figure 2c) in *P. rhoeas* and in phenological traits of both species (figure 2d,e; electronic supplementary material, table S4). In *O. viciifolia*, the probability of seed production was lower in ancestors exposed to M, while no differences existed in descendants (figure 2b). Similarly, ancestors of *P. rhoeas* exposed to late M produced lighter seeds than those exposed to late L (figure 2c) and those exposed to early M produced heavier seeds than those exposed to early L (electronic supplementary material, figure S4), while in descendants, no significant differences existed between treatment levels (figure 2c, electronic supplementary material, figure S4). Thus, in both species, transgenerational responses cancelled out treatment effects on fitness-related traits detected in ancestors.

There existed a significant early treatment \times PTR interaction on time to flowering of *O. viciifolia* (figure 2d; electronic supplementary material, table S4). In ancestors, no significant treatment differences existed, while descendants exposed to L flowered significantly earlier than those exposed to M. Moreover, significant three-way interactions including year, PTR and treatment show that the strength of the transgenerational response varied among years (electronic supplementary material, table S4). In time to the emergence of *P. rhoeas* (figure 2e), no significant transgenerational response existed in G_1 , because no significant differences existed between ancestors and descendants in 2013 (figure 2e). However, significant transgenerational responses existed in G_2 and G_3 , given the differences between ancestors and descendants in 2014 and 2015. Within-treatment contrasts show that the speed of the response was faster in L than in M (figure 2e). In L, G_2 emerged significantly earlier than G_1 , while in M, significant differences existed later, between G_2 and G_3 (figure 2e). A significant early \times late treatment \times PTR interaction existed on

the number of seeds produced by *O. viciifolia* (figure 2f; electronic supplementary material, table S4). While in MM and LL, descendants had higher average seed production than their ancestors, the reverse pattern existed in ML and LM (figure 2f), suggesting that the inter-seasonal predictability affected the transgenerational response.

In the reciprocal transplant experiment, the interactions between matriline treatment and descendant treatment were not significant in any of the traits measured in *O. viciifolia* and *P. rhoeas* (all interactions: $\chi_9^2 \leq 14.9$; $p \geq 0.1$). In these analyses, the power to detect a treatment effect in descendants of the effect size observed in ancestors was greater than 99%.

4. Discussion

We experimentally tested whether differences in intrinsic precipitation-predictability affect life-history traits and population growth of two plant species and whether intrinsic precipitation-predictability triggers transgenerational responses. Under less predictable precipitation, both plants exhibited accelerated phenology, but contrary to expectations, reproductive success and population growth were positively affected. Both species exhibited transgenerational responses that mitigated treatment effects detected in ancestors.

Advanced phenology in less predictable conditions (figure 1a–d) is in line with our first prediction and theory that lower predictability should favour earlier reproduction [6]. *Onobrychis viciifolia* exposed to less predictable precipitation produced more seeds (electronic supplementary material, table S1; figure 1f), and *P. rhoeas* exposed to less predictable precipitation produced heavier seeds (electronic supplementary material, table S1; figure 1e) that will have higher reproductive success than lighter seeds (electronic supplementary material, table S3; see ‘initial seed mass’ effect on ‘ N seeds produced’). Differences in the response to precipitation-predictability between the two species may have arisen owing to their different life-history strategies. While *O. viciifolia* exhibits a K -strategy (high investment in seed quality), *P. rhoeas* exhibits an r -strategy (high investment in seed quantity; [6]). Thus, our results suggest that, to increase their reproductive success, both plants invested in the ‘non-priority’ trait (*O. viciifolia* in seed number; *P. rhoeas* in seed quality), which is congruent with earlier beliefs that ‘no organism is completely ‘ r -selected’ or completely ‘ K -selected’, but all must reach some compromise between the two extremes’ [6]. This also suggests that both species first invested in the trait determining the plant’s strategy (*O. viciifolia* in seed size and *P. rhoeas* in seed number) and, if possible, also in the ‘non-priority’ trait. In addition to the above-mentioned effects on fitness-related traits, in both species, the probability of reproducing increased under less predictable conditions (electronic supplementary material, table S1). Consequently, in both plant species, earlier reproduction was associated with an increase in reproductive success, immediate population growth (increased per capita growth rate) and future population growth (in *P. rhoeas* owing to the treatment effect on seed mass and its effect on seed production; electronic supplementary material, table S3). These results contradict predictions from theoretical models (prediction (ii)), i.e. that earlier reproduction reduces the investment in individual offspring or offspring number [7,8], and they provide experimental support for the

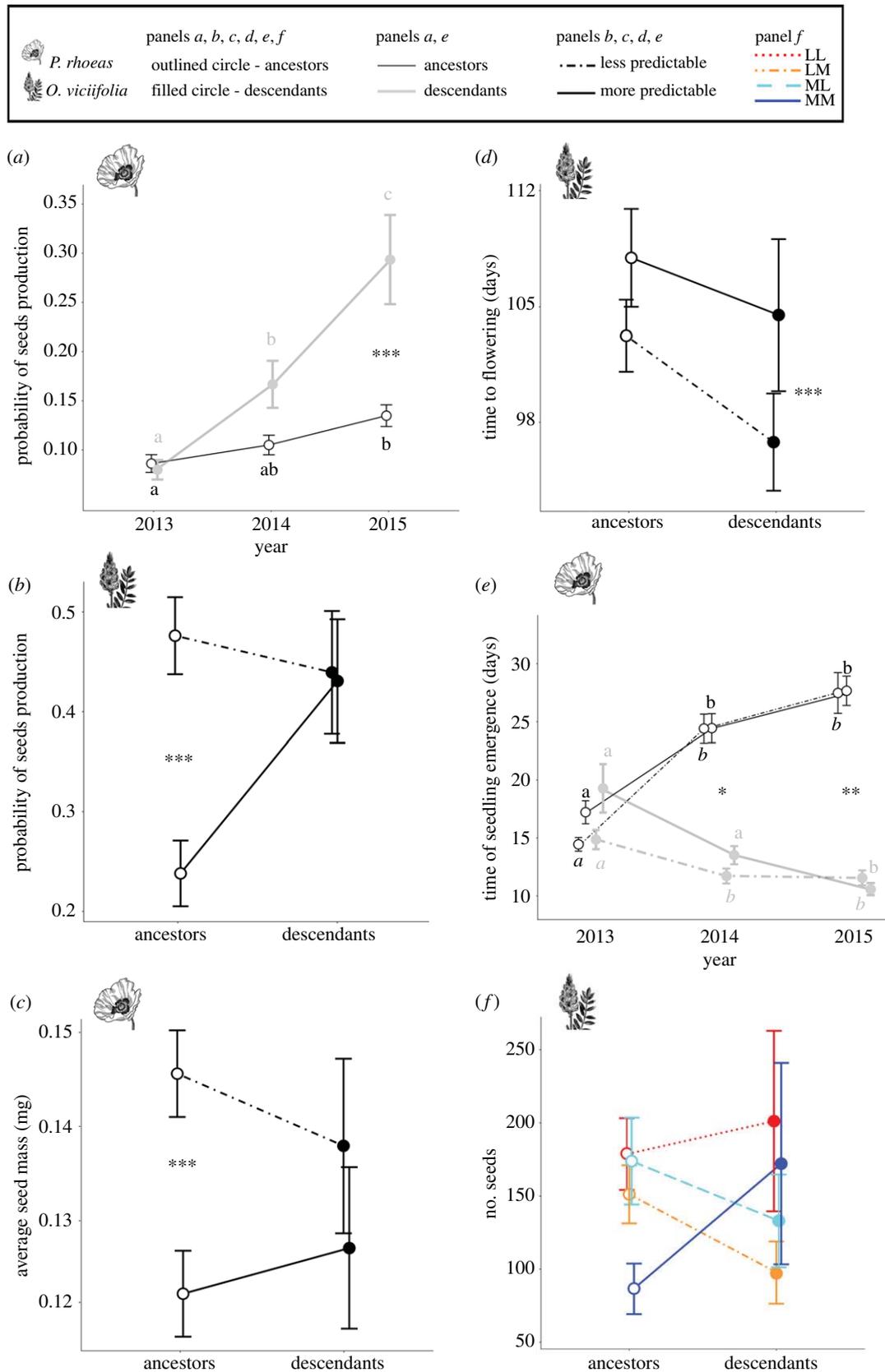


Figure 2. Transgenerational responses of *P. rhoeas* and *O. viciifolia*. Significant interactions of year and/or treatments (early and/or late treatment) with PTR (ancestors versus descendants) are shown (electronic supplementary material, table S4). (a) Significant treatment-unrelated transgenerational response of *P. rhoeas* on the probability of producing seeds. (b) Effects of early treatment on the transgenerational response in the probability of seed production of *O. viciifolia*. (c) Effects of late treatment on the transgenerational response in the average seed mass of *P. rhoeas*. (d) Effects of early treatment on the transgenerational response in the time to flowering of *O. viciifolia*. (e) Significant early treatment \times year \times PTR interaction on the time of seedling emergence of *P. rhoeas*. Significant within-year contrasts between ancestors and descendants are indicated with asterisks $*0.05 > p \geq 0.01$; $**0.01 > p \geq 0.001$; $***p < 0.001$ and significant between-year contrasts within treatment levels with letters. Grey letters are used for descendants and black letters for ancestors. Italicized letters correspond to the L and normal letters to the M treatment. (f) Significant early treatment \times late treatment \times PTR interaction on the number of produced seeds by *O. viciifolia*. (Online version in colour.)

hypothesis that variable and less predictable environments can increase population growth rates [4,24].

As expected (prediction (iii)), both plant species exhibited rapid transgenerational responses with respect to the simulated precipitation-predictability (electronic supplementary material, table S4; figure 2b–f). In most cases, treatment-induced differences were independent of the year (figure 2b–d,f), showing that transgenerational responses affected each descendant generation similarly (G_{1-3}). In the reciprocal transplant experiment, matriline treatment \times descendant treatment interactions were not significant, which contrasts to prediction (iv): that descendants of a given matriline will perform better in the matriline's treatment than descendant of matriline of the other precipitation treatments. This suggests that transgenerational responses to precipitation-predictability are not the result of local adaptation [18]. The high power for detecting a significant interaction, together with the significant treatment effects on matriline ancestors and ancestors planted in the same year and plot (figure 1), suggests that the detected transgenerational responses changed the phenotypic plasticity.

Transgenerational responses with respect to the precipitation-predictability were mostly immediate, because they mitigated treatment effects already in the first descendant generation (figure 2b,c; electronic supplementary material, figure S4). However, there was one exception: transgenerational responses shifted time to seedling emergence in *P. rhoeas* over more than one generation (figure 2e). Descendants exposed to L exhibited shorter time to emergence in G_2 (compared to G_1), while in M, time to emergence decreased not until G_3 (figure 2e). This shows that the speed of the response depended on the precipitation-predictability regime experienced during early growth [25]. There was a significant early \times late treatment \times PTR interaction on the number of seeds produced by *O. viciifolia* (figure 2f). In MM and LL, the average seed number increased from ancestors to descendants, and it decreased in ML and LM (figure 2f). This suggests that transgenerational responses to a given predictability regime may depend on inter-seasonal predictability and not only on high transgenerational predictability. Interestingly, the effects of inter-seasonal predictability only existed in this trait, suggesting that in *P. rhoeas* and *O. viciifolia* inter-seasonal predictability might be of lower importance. Similarly, while precipitation-predictability during early growth induced rapid transgenerational responses, during late growth precipitation-predictability only induced a single transgenerational response on average seed mass of *P. rhoeas* (figure 2c; electronic supplementary material, table S4). Given that in ancestors of both plant species, many traits were affected by late treatment (figure 1c–f; electronic supplementary material, table S1), this suggests that transgenerational responses mainly exist with respect to early growth conditions, when plants are most sensitive to environmental changes [26]. This indicates that differences in precipitation-predictability during late growth may affect plants most, because responses could be more difficult or

take longer, potentially enhancing extinction risk and reducing population persistence [4], both putting species at risk. The detected transgenerational responses to precipitation-predictability are in line with theoretical models that identified environmental predictability as a key parameter for how organisms respond to environmental change [9].

In conclusion, our study experimentally demonstrates that low short-term predictability of precipitation causes an advance in plant phenology and an increase in reproductive success, and population growth. In contrast to expected negative impacts of lower precipitation-predictability, our study suggests that at least some plant species may benefit from lower precipitation-predictability. Plants also exhibited rapid transgenerational responses that mitigated treatment effects observed in ancestors, which may have changed phenotypic plasticity, in line with theoretic models showing that less predictable environments may favour the evolution of phenotypic plasticity [11,12]. This suggests that low predictability of precipitation events *per se*, at least as long as no extreme events occur, may not be as damaging as forecasted. However, transgenerational responses mainly existed with respect to early growth conditions, suggesting that during early life, transgenerational responses to precipitation-predictability might be easier. These findings demonstrate that the degree of intrinsic predictability *per se* cannot predict effects on ecology, and evolution, as different types of predictability (short-term, seasonal, and transgenerational predictability) may affect organisms differently. Therefore, to anticipate the impact of climate change, theoretical models and mitigation efforts should consider the effects of different types of intrinsic precipitation-predictability on the capacity of species responses to new weather situations. Moreover, our results also suggest that subtle changes in weather predictability may not be as harmful as forecasted by global change scenarios.

Authors' contributions. P.S.F. and M.M.-S. designed the study. M.M.-S. implemented the study and collected the data and samples under the supervision of P.S.F. M.M.-S. analysed the data with inputs from P.S.F. and M.v.K.; M.M.-S. and P.S.F. drafted the manuscript, and all authors commented on the following versions of the manuscript.

Competing interests. We declare we have no competing interests.

Funding. Funding was provided by the Spanish Ministry of Economy and Competitiveness (grant no. CGL2012-32459 to P.S.F.), Ministerio de Ciencias, Investigación, y Universidades (grant no. CGL2016-76918 AEI/FEDER, UE to P.S.F.) and the Swiss National Foundation (grant nos. PPOOP3_128375, PP00P3_152929/1 to P.S.F.). M.M.-S. was supported by a PhD grant (grant no. BES-2013-062910) financed by the Spanish Ministry of Economy and Competitiveness and a travel grant of the Universidad Rey Juan Carlos.

Acknowledgements. We thank Guillermo Mercé, Blanca Santamaría, Diana Íñigo, Miguel Moreno, María Urieta, Helena Clavero, María Jesús Pueyo, José Azorín, Federico Fillat, Ricardo García, Luis Villar, Marc Stift, Gemma Palomar, Guillem Masó and Emily Haeuser for help and suggestions.

References

1. Stocker TF *et al.* 2013. IPCC, 2013: Climate change 2013: the physical science basis. In *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press.
2. Pennkamp F *et al.* 2019. The intrinsic predictability of ecological time series and its potential to guide forecasting.

- Ecol. Monogr.* **89**, e01359. (doi:10.1002/ecm.1359)
3. Sala OE *et al.* 2000 Global biodiversity scenarios for the year 2100. *Science* **287**, 1770–1774. (doi:10.1126/science.287.5459.1770)
 4. Ashander J, Chevin L-M, Baskett ML. 2016 Predicting evolutionary rescue via evolving plasticity in stochastic environments. *Proc. R. Soc. B* **283**, 20161690. (doi:10.1098/rspb.2016.1690)
 5. Dewar RE, Richard AF. 2007 Evolution in the hypervariable environment of Madagascar. *Proc. Natl Acad. Sci. USA* **104**, 13 723–13 727. (doi:10.1073/pnas.0704346104)
 6. Pianka ER. 1970 On r- and K-selection. *Am. Nat.* **104**, 592–597. (doi:10.1086/282697)
 7. MacArthur RH. 1972 *Geographical ecology: patterns in the distribution of species*. Princeton, NJ: Princeton University Press.
 8. Einum S, Fleming IA. 2004 Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. *Evol. Ecol. Res.* **6**, 443–455.
 9. Botero CA, Weissing FJ, Wright J, Rubenstein DR. 2015 Evolutionary tipping points in the capacity to adapt to environmental change. *Proc. Natl Acad. Sci. USA* **112**, 184–189. (doi:10.1073/pnas.1408589111)
 10. Merilä J, Hendry AP. 2014 Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl.* **7**, 1–14. (doi:10.1111/eva.12137)
 11. Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007 Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* **21**, 394–407. (doi:10.1111/j.1365-2435.2007.01283.x)
 12. Reed TE, Waples RS, Schindler DE, Hard JJ, Kinnison MT. 2010 Phenotypic plasticity and population viability: the importance of environmental predictability. *Proc. R. Soc. B* **277**, 3391–3400. (doi:10.1098/rspb.2010.0771)
 13. Marshall DJ, Burgess SC. 2015 Deconstructing environmental predictability: seasonality, environmental colour and the biogeography of marine life histories. *Ecol. Lett.* **18**, 174–181. (doi:10.1111/ele.12402)
 14. Karl TR, Knight RW, Plummer N. 1995 Trends in high-frequency climate variability in the twentieth century. *Nature* **377**, 217–220. (doi:10.1038/377217a0)
 15. Beier C *et al.* 2012 Precipitation manipulation experiments: challenges and recommendations for the future. *Ecol. Lett.* **15**, 899–911. (doi:10.1111/j.1461-0248.2012.01793.x)
 16. Siepielski AM *et al.* 2017 Precipitation drives global variation in natural selection. *Science* **355**, 959–962. (doi:10.1126/science.aag2773)
 17. Christensen JH *et al.* 2013 Climate phenomena and their relevance for future regional climate change. In *Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change* (eds TF Stocker *et al.*). Cambridge, UK: Cambridge University Press.
 18. Kawecki TJ, Ebert D. 2004 Conceptual issues in local adaptation. *Ecol. Lett.* **7**, 1225–1241. (doi:10.1111/j.1461-0248.2004.00684.x)
 19. Masó G, Ozgul A, Fitze PS. In press. Decreased precipitation predictability negatively affects population growth through differences in adult survival. *Am. Nat.* (doi:10.1086/706183)
 20. Carbonero CH, Mueller-Harvey I, Brown TA, Smith L. 2011 Sainfoin (*Onobrychis viciifolia*): a beneficial forage legume. *Plant Genet. Resour.* **9**, 70–85. (doi:10.1017/S1479262110000328)
 21. R Development Core Team. 2016 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
 22. Gotelli NJ, Ellison AM. 2013 *A primer of ecological statistics*, 2nd edn. Sunderland, MA: Sinauer Associates Inc.
 23. Faul F, Erdfelder E, Lang A-G, Buchner A. 2007 G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **39**, 175–191. (doi:10.3758/BF03193146)
 24. Lawson CR, Vindenes Y, Bailey L, van de Pol M. 2015 Environmental variation and population responses to global change. *Ecol. Lett.* **18**, 724–736. (doi:10.1111/ele.12437)
 25. Gomez-Mestre I, Jovani R. 2013 A heuristic model on the role of plasticity in adaptive evolution: plasticity increases adaptation, population viability and genetic variation. *Proc. R. Soc. B* **280**, 20131869. (doi:10.1098/rspb.2013.1869)
 26. Burton T, Metcalfe NB. 2014 Can environmental conditions experienced in early life influence future generations? *Proc. R. Soc. B* **281**, 20140311. (doi:10.1098/rspb.2014.0311)
 27. March-Salas M, van Kleunen M, Fitze PS. 2019 Data from: Rapid and positive responses of plants to lower precipitation predictability. Dryad Digital Repository. (<https://doi.org/10.5061/dryad.s558287>)