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2 **Joint impact of competition, summer precipitation, and maternal effects on**
3 **survival and reproduction in the perennial *Hieracium umbellatum***

4

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Abstract

Most studies on consequences of environmental change focus on evolutionary and phenotypic plastic responses, but parental effects represent an additional mechanism by which organisms respond to their local environment. Parental effects can be adaptive if they enhance offsprings ability to cope with environments experienced by their parents, but can also be non-adaptive for instance when offspring from benign environments are just better provisioned and hence perform better than offspring from less benign environments. Parental effects originate from both the abiotic and biotic environmental variation. However, the effects of the parental abiotic and biotic environment are rarely studied together. We make use of an experimental set-up containing plots in a natural heath land, where summer precipitation was manipulated to reflect either ambient or drought conditions. In both plot types, competition from grasses was prevalent. We assessed survival and reproduction of *Hieracium umbellatum* offspring originating from ambient and drought plots grown in a factorial design with two levels of moisture (control and drought) and two levels of competition (grown with and without a local perennial grass). The maternal environment strongly affected offspring performance. Biomass and reproduction was higher in offspring from ambient plots in agreement with the hypothesis of a better maternal provisioning in the most benign environment. However, adding competition revealed potentially adaptive responses to survival, and altered allocation to reproduction in offspring from maternal drought plots. Under combined competition and drought (mimicking maternal drought plots), survival was only reduced in offspring from ambient plots, and offspring from drought plots survived best. When grown in competition under control watering conditions mimicking maternal ambient plots, offspring from drought plots (growing in an environment different from their maternal one) showed a 25% reduction in reproduction. Potential adaptive responses to the home

43 maternal environment were only revealed when jointly manipulating levels of competition and
44 water availability.

45

46

47 **Keywords:** Adaptation, abiotic and biotic environment, maternal provisioning, plant ecology

Introduction

Evolutionary responses to local environments can happen fast, and sometimes over the course of relatively few generations (Carroll et al 2007). While responses to local environments are often considered to be the result of microevolutionary changes, several studies also found that a portion of the phenotypic changes observed in response to environmental change can be attributed to transgenerational effects, where the parental environment influences offspring performance (Roach and Wulff 1987; Marshall and Uller 2007; Bossdorf et al 2008). In such cases, parental effects represent an additional component of phenotypic variation that has the potential to boost organism's capacity to cope with environmental change (Bossdorf et al 2008). Parental effects might manifest themselves as parents in high resource environments provisioning their offspring better and produce larger or faster growing offspring than parents in low resource environments (maternal provisioning) (Stearns 1992; Mousseau and Fox 1998; Huey et al 1999). Parental effects can also alter expression of genes in their offspring resulting in short-term heritable variation beyond the mere allelic effects of transmitted genes (Herman and Sultan 2016). These effects may be adaptive when they enhance the offspring's ability to cope with environments that are similar to the environment experienced by their parents (Galloway 2005) (a transgenerational equivalent to the beneficial acclimation hypothesis *sensu* Huey et al 1999). However, empirical studies found that transgenerational effects can either enhance or decrease offspring performance in parental environments, questioning to what degree transgenerational effects are generally adaptive (Marshall and Uller 2007; Herman and Sultan 2011; Walther et al 2016).

70 Parentally transmitted environmental effects may be particularly important for plants where,
71 because of their sessile nature, a strong temporal autocorrelation between the maternal and
72 offspring environments is expected and parental adult environment might be more predictive of
73 the offspring adult environment than the offspring early life environment (Auge et al. 2017).
74 Transgenerational effects enable response to abiotic environmental factors such as drought and
75 heat stress (Marshall and Uller 2007; Walther et al 2016), and light environment (Galloway and
76 Etterson 2007). Besides abiotic factors, transgenerational effects attributable to variation in the
77 parental biotic environment are also documented. For example, phenotypic variation for fitness
78 traits in offspring can be caused by variation in the levels of competition experienced by their
79 parents (Violle et al 2009), levels of herbivory (Agrawal 2001; terHorst and Lau 2011), and
80 species diversity in the parental plant community (Rottstock et al 2017). To better understand the
81 fitness consequences of transgenerational effects, offspring performance should be evaluated in a
82 realistic ecological setting reflecting both the abiotic and biotic parental environment. Although
83 transgenerational effects driven by variation in both abiotic and biotic parental environment have
84 been reported, their effects have rarely been studied together (but see e.g. Metz et al 2015).
85 In this study, we use a replicated climate change experiment with ambient (control) and summer
86 drought treatments established under natural conditions in a species-rich dry heath land (EU
87 Climoor and Vulcan projects, Beier et al 2004). We study the effects of the maternal
88 environment on offspring performance in the short-lived perennial herb *Hieracium umbellatum*.
89 The effect of maternal environment (ambient or drought) on offspring performance may be a
90 result of both microevolutionary and transgenerational effects. However, given a very close
91 proximity of experimental plots with no barrier to pollen and seed flow, combined with a recent

establishment of our study plant in these plots, we consider differences in offspring performance between maternal environments to be mostly caused by transgenerational effects. Given a maternal environmental effect, we expected seeds collected from maternal plants growing in drought and in ambient experimental plots to differ. Specifically, we tested two non-mutually exclusive hypotheses (Monaghan 2008): According to the maternal provisioning hypothesis (hypothesis a), offspring produced in the most benign environment (here ambient plots) perform better than offspring produced in the least benign environment (drought plots) especially when grown in less benign environments. According to the transgenerational beneficial acclimation hypothesis (hypothesis b), offspring perform best in the environment matching their maternal environment. In other words, when growing in ambient conditions, offspring from maternal ambient environment should perform better than offspring from maternal drought environment and vice versa when growing in drought conditions, offspring from maternal drought environment should perform better than offspring from maternal ambient environment. To add a biotic component, *H. umbellatum* offspring performance was investigated when grown either alone or in competition with a locally common perennial grass. We hypothesized that the addition of a competitor would increase environmental stress to the focal plants (i.e. through competition for space, water, and/or nutrients) and therefore exacerbate the detrimental effect of growing either in the least benign (drought) environment (hypothesis a) or the non-maternal environment (hypothesis b). We note that responses consistent with the transgenerational beneficial hypothesis would also be consistent with adaptation through microevolutionary changes in each maternal environment.

Material and Methods

Sampling site and experimental climate manipulation

The sampling site was a dry grass/heath land located in East Jutland, Denmark (Mols). The vegetation was dominated by the dwarf shrub *Calluna vulgaris* and the perennial grass *Deschampsia flexuosa*. A number of perennial and a few annual herbs co-occur with these two dominant species. The soil was sandy podzol with a pH of approximately 3.9, and water table was below the root zone of the plants (Sowerby et al 2008). Experimental manipulation of climate, consisting of three plot types (ambient, drought and warming) each consisting of three replicated 20 m² (size 4×5 m) plots, was initiated in 1999. In this study, we only used seeds collected from plants growing in ambient and drought plot. Light scaffolding was built around all plots to ensure that any impact of scaffolding occurred in all treatments (Fig. 1). Drought treatment was put into practice by rain exclusion for two months every year in the spring/summer growing season. This was achieved by an automated system extending a transparent waterproof material over the drought treatment plots creating a roof during rain events greater than 0.2 mm. These plot covers were retracted immediately after a rain event. Outside the period with rain exclusion, the soil moisture in drought plots were identical to ambient plots. The drought treatment was found to transiently decrease soil respiration but this recovered after the drought treatment had ceased (Sowerby et al 2008). Further details of location and experimental set up are given in Beier et al (2004), and Sowerby et al (2008). Soil temperature and soil moisture (Table 1) was measured using in-situ thermocouple and time domain reflectometry sensors that were installed 5 cm below the soil surface, and data was recorded on an hourly basis using CR1000 data loggers (Campbell Scientific Ltd, UK).

Both ambient and drought plots had a dense vegetation cover, and these two plot types did not differ in annual above ground biomass accumulation and plant species richness (Penuelas et al

2007). Over the last decade perennial grasses have increasingly dominated the site especially after a heather beetle attack, *Lochmaea suturalis* (Thomson), in 2003, which killed many *Calluna* shrubs. Root biomass in the soil was high but not different between control and drought plots (around 250 g dry weight m⁻², Holmstrup et al 2013). Thus, both in control and drought plots, plants experienced increasing competition from grasses.

Study species and sampling

None of the herbs at the study site was present in high frequencies relative to *Calluna* shrubs and grasses. The perennial herb *Hieracium umbellatum* (Asteraceae) was found in all three ambient and three drought plots and was used as model plant for maternally inherited responses to the biotic and abiotic environment. *Hieracium umbellatum* flowers from June to August. Fruits were collected in 2013 in late September from a total of 12 maternal plants in the ambient plots (4 families per plot) and 10 maternal plants in the drought plots (as in one plot only two individuals could be sampled). Fruits collected from the same maternal plant represented a maternal family. Fruits were collected from maternal plants that grew at least one m. apart (to minimize the probability of sampling the same individual twice) and kept dry in paper bags until use.

Since 1999, the vegetation in the experimental plots was monitored regularly, providing yearly estimates of the frequency of all plant species present in each individual plot (Penuelas et al 2007; Kappel Schmidt (unpublished data)). This showed a continuous but low presence (ca. 4 % of total plant cover) of *H.umbellatum* across years, except for 2009 (ambient), 2010 (drought), and 2012 (both ambient and drought) where the species was either absent, or almost absent from these plots, however, it was continuously present in the surrounding vegetation. In comparison,

160 the grass *D. flexuosa* was consistently present in all plots with an average cover of 74 % in
161 ambient and 79 % in drought plots (Kappel Schmidt, unpublished data).

162 The experimental plots with their open sided scaffolding and close proximity to each other (Fig.
163 1) allow pollen and seed influx from neighboring plots and the surrounding natural vegetation.
164 Given the recorded absence of *H. umbellatum* from both ambient and drought plots in some
165 years, the maternal plants we used were likely established in the plots within 1-4 years prior to
166 our seed sampling in 2013.

167

168 Greenhouse experiment

169 In March 2014, seeds from each maternal plant were sown in individual seed trays. In early May,
170 sixteen seedlings per maternal line were planted into individual pots (pot height 30 cm; pot
171 diameter 20 cm). Care was taken to choose similar sized seedlings from each of the maternal
172 families.

173 The soil used in the pots was collected from one location at the experimental site at Mols situated
174 just outside the climate treatment plots, and brought to the green house in six 200 liter plastic
175 containers. When soil was excavated top soil (top 0-10 cm) and deeper soil (10-30 cm depth)
176 were collected in separate plastic containers. Large roots and stones were removed by hand
177 before individual pots were filled. We placed a layer of small stones in the bottom of the pots
178 and filled first with a 15 cm layer of soil collected from 10-30 cm depth and then with a 10 cm
179 layer of topsoil (0-10 cm).

We used a factorial combination with two levels of water treatment (control (ambient) and drought) and two levels of competition treatment (with or without a competitor). This resulted in four treatment combinations (Control water treatment, Drought, Competition and Control water treatment, Competition and Drought). Every maternal line was represented by four seedlings grown in each of these four treatment combinations.

Competition was applied by adding one individual of the perennial grass *Deschampsia flexuosa* to the pot together with the *H. umbellatum* seedling. *Deschampsia flexuosa* was obtained by digging up a few large (0.5 m diameter) *D. flexuosa* tussocks growing just outside the experimental plots. In the lab, tussocks were separated into similar sized units (4-5 leaves with accompanying roots) and used as competitor. If any plants died within a week after transplantation it was replaced. After one week we assumed that death of plants due to transplanting was no longer an issue, and the experiment began.

Watering treatment

Water was applied automatically from a sprinkler system installed above the tables twice a day. Each watering event consisted of 6 minutes watering of control pots and two minutes watering of drought pots. These watering levels were adopted to mimic soil moisture values measured in the field experiment. Soil moisture analysis from the field experimental plots (Table 1) showed that during the summer, the soil moisture in drought plots was approximately one third to half of the soil moisture in the control plots. While we could not mimic the exact soil moisture, we aimed to mimic the relative difference in water availability. What we refer to as drought treatment received approximately one third of the water than the control treatment received. We noted that

plants in the drought treatment showed leaves with clearly reduced water tension in between watering was applied confirming dry condition, and this was not observed for plants in the control water treatment.

Due to space constraints, it was not possible to fit all plants in a single greenhouse cell, and pots were placed in two adjacent greenhouse cells. In each cell, pots were placed on two tables that were separated by a transparent plastic curtain from the ceiling to the floor. One table received the drought water treatment and the other the ambient (control) water treatment. During the entire course of the experiment (May-October) pots within tables were randomized twice a week to avoid any position effects.

Validation of water treatment

After the experiment had ended and all above ground biomass was harvested, we arbitrarily chose 60 pots; 30 pots from the control water treatment and 30 from the drought treatment. These pots were left to dry for 1 month after which they were weighed. Pots were then watered according to their respective treatment, and subsequently weighed approximately one hour after watering was applied. We used the difference in weight between wet and dry pots to test that pots assigned to the drought treatment did indeed contain less water than control pots. Difference in weight between pots subject to drought and control watering was roughly 1/3 (weight difference between watered and dry pots in control watering treatment: 56.8 ± 6.7 g and weight difference pots drought treatment: 21.6 ± 6.8 g, t-test: $t = 3.67$, $df = 1$, $P = 0.0005$).

223 Plant traits

224 Weight of seeds collected from maternal plants in the experimental plots were obtained from 18
225 of the seed families used (9 from ambient and 9 from drought plots). Between 10 and 20 seeds
226 from each maternal plant were weighed together using a 0.01 mg precision balance, and used to
227 estimate average weight of an individual seed.

228 Onset of flowering was checked every three days from end of June and until all plants had
229 flowered. At the end of the experiment, we recorded survival and harvested above ground
230 biomass of all individuals (including those that had died). The above ground biomass was
231 divided into vegetative and reproductive (all flowers and fruits) and kept in individual paper
232 bags. Bags were placed for five days in ovens heated to 70 °C and dry weight of vegetative (all
233 leaves and stems) and reproductive biomass (flowers and fruits) was estimated for each
234 individual plant using a 0.01 g precision balance.

235 It was not feasible to collect the roots for belowground biomass, as the high fraction of roots and
236 organic material present in the field soil had grown entangled with the focal plant root making
237 below ground biomass very error prone to give reliable root biomass estimates.

238 Since plants produced flowers and fruits continuously across the growing season many plants
239 that did not survive till the end of the experiment would still have produced some flowers and
240 even fruits. We sampled fruits and flowers from all plants (also those that had died), hence even
241 if plants did not survive the entire growing season they could still have some reproduction.

242

243 Statistical analysis

244 We used linear mixed models to analyze offspring response variables (aboveground biomass,
245 onset of flowering, number of flowers, flower weight) by considering the effect of water
246 treatment (control vs. drought), the effect of competition (presence or absence of competitor), the
247 maternal environment (ambient or drought plots) and their interactions. All these factors were
248 treated as fixed effects. In addition, we also included the effect of maternal family nested within
249 each maternal environment and these were treated as random. The experiment was performed in
250 two greenhouse cells (because space was not sufficient to contain all pots in one cell) and
251 accordingly we also added a (random) cell effect to our models. Because of experimental
252 constraint, watering conditions were manipulated for a whole table within each cell and as a
253 consequence watering treatment was considered as a “whole plot” effect nested within each cell
254 and we analyzed the design as a split plot design.

255 The corresponding mixed model was fitted using the functions lmer (for all biomass and
256 reproductive traits) implemented in the R package lme4 (Bates et al 2015). If significant
257 interactions between maternal origin and any of our applied treatments (competition and water)
258 were detected, we separately analyzed the data from maternal ambient and maternal drought
259 plots to examine how treatment differed between offspring from different maternal origin.

260 The variables biomass, flower number and weight, onset of flowering were analyzed using
261 linear models. Test of significance of fixed factors were obtained from F-test using
262 Satterthwaite’s approximation and type III sum of squares from the anova table. Test of
263 significance for random effects were obtained via likelihood ratio tests comparing models with/
264 without the random effect.

Flower number was log-transformed prior to analysis to meet the linear model assumption of normally distributed errors.

Survival was analyzed using a generalized linear model (hereafter glm), assuming a binomial distribution and using the logit link function. The survival data was fitted using glm model assuming the same design as the mixed model used for the other traits (function glmer in lme4). In this case, tests of significance for each model component were performed using likelihood ratio tests for comparing models with/ without each effect. We first fitted a full model with 3 ways interactions and then compared the fit with reduced models containing all or a subset of all two ways interactions (Bates et al 2015). Accordingly, in the results section, we report the change in deviance attributed to each factor and the corresponding p-value.

All analysis was performed in R (version 3.4.2)

Results

The mean weight of individual seeds collected from maternal plants in the field did not differ between ambient and drought plots, albeit a tendency for the seeds collected in the ambient plots to be heavier (mean seed weight (mg) ambient: 0.044 ± 0.003 , drought 0.038 ± 0.003 , $F_{1,17} = 1.77$, $P = 0.2$).

Offspring survival

The watering treatment had a strong impact on survival with the highest mortality for plants grown in drought treatment (Dev=5.62, $P = 0.0178$, Fig 2A). No significant three way interaction was detected (Dev=0.93, $P = 0.34$), but there were significant interactions between Maternal

environment and Competition (Dev=5.07, P=0.0243), and between Competition and Water treatment (Dev=5.62, P=0.018). Analyzing the maternal environment separately revealed significant effects of water treatment on survival of offspring from both maternal environments (Ambient origin; Dev= 54.41, $P < 0.0001$, Drought origin; Dev 5.99, $P = 0.014$), but a significant interaction effect between competition and water treatment was only detected in offspring from the maternal ambient environment (Ambient origin; Dev= 4.94, $P = 0.026$; Drought origin; Dev=0.96, $P = 0.30$). When growing without a competitor in the greenhouse drought treatment, offspring originating from maternal ambient environment survived better than offspring from maternal drought environment. When grown with a competitor, survival decreased for offspring originating from maternal ambient plots (Figure 2A, dashed grey lines), but this decrease was not found in offspring originating from maternal drought plots (Fig 2A, dashed black lines).

Plants originating from maternal ambient environment showed a significant interaction between maternal family and competition (Dev= 4.87, $P=0.027$), but not between maternal family and water treatment (Dev = 2.15, $P = 0.14$, Fig. 3A grey lines). However, for plants originating from maternal drought environment, no interaction effect between maternal family and either competition or water treatment was detected (Dev = 0.01, $P = 0.99$, Dev = 0.01, $P = 0.99$, respectively; Fig. 3A black lines).

Biomass of offspring

Maternal environment, Water treatment and Maternal family all affected total aboveground biomass (vegetative plus reproductive tissue) and vegetative biomass (Table 2).

Total biomass was lower for plants grown under the greenhouse drought treatment, and more so for plants originating from maternal drought plots (Fig. 4). No interactions between these factors were detected (Table 2), suggesting that the impact of Water treatment and Competition on above grounds biomass was similar for plants originating from drought and ambient plots.

Reproduction of offspring

We found no difference in onset of flowering between any of the treatments or maternal environments (Table 2). Because flower weight and flower number were highly correlated (pairwise correlations: Ambient origin: $r = 0.87$, $P < 0.001$, Drought origin: $r = 0.94$, $P < 0.001$), we only present the results for flower number (Table 2). Plants originating from the maternal ambient plots produced more flowers than plants from the maternal drought plots (Table 2, Fig. 2B). Irrespective of maternal origin, plants produced more flowers in the control water treatment than in the drought treatment (Table 2, Fig 2B).

The impact of competition on flower number varied with Water treatment (Competition x Water treatment, Table 2). In the drought treatment, competition did not affect flower number (Fig. 2B dashed lines), but in the control water treatment, plants in competition produced less flowers than plants grown without a competitor (Fig 2B, solid lines), notably plants originating from maternal drought environment showed 25% decrease in flower production under competition (Fig 2B, solid black line)

A significant effect of maternal family was found for both maternal environments (Ambient origin: $\text{Dev}=15.4$, $P < 0.00001$, Drought origin: $\text{Dev}=13.1$, $P= 0.0003$, Fig. 3B), but no interaction between Maternal family and treatments was found in either maternal environment.

329

330 **Discussion**

331 We studied phenotypic responses in offspring grown in greenhouse environments that mimicked
332 different combinations of their maternal abiotic and biotic environment. The main results were
333 that the maternal environment had a strong impact on offspring performance, and that
334 interspecific competition changed the phenotypic response to water treatment for offspring
335 survival and reproduction depending on which maternal environment they originated from.

336 The greenhouse drought treatment had the largest effect on offspring performance. Overall,
337 plants survived less well, had lower above ground biomass and produced fewer flowers when
338 grown in drought conditions relative to control. In contrast, effect of competition (on survival
339 and reproduction) was only revealed through its interactions with either water treatment or
340 maternal environment.

341 All maternal families used in this experiment originated from the same heathland population and
342 were collected within a small geographic area (Fig. 1). The close geographic proximity of
343 experimental plots from which fruits were collected and the open sided scaffolding allowed for
344 gene flow (both pollen and seed) among plots and the surrounding natural vegetation. The
345 difference in levels of water availability during the growing season are therefore the most likely
346 driver of the differences in performance between offspring originating from ambient and drought
347 plots. Our design does not allow us to formally test if the differences in offspring response we
348 observed were mostly due to maternal effects or to microevolutionary differences in response to
349 selection between ambient and drought plots. The experiment we used as source for the maternal
350 families used here had been running for 14 years prior to our sampling. This could allow for

differential response to selection on plants growing in drought versus ambient plots. Indeed, fast evolutionary response to environment changes has been previously documented (e.g. Franks et al 2007; Cheptou et al 2008; Shaw & Etterson 2012; Bataillon et al 2016), and the significant differences between maternal families indicate the presence of genotypic variation and therefore potential for responding to selection in each parental environment. However, besides the swamping from gene flow from other plots and from outside plots, the fact that our study plant was absent – and close to absent - from the plots in 2009 (ambient), 2010 (drought) and 2012 (both) undermines the idea that evolutionary response can account for the differences we uncovered. The maternal plants from which we sampled seeds in 2013 must have very recently (re)established in the plots, thus leaving very little time for cumulative selection to generate genetic differences among plots. Seed germination and initial rosette formation occur in early spring before drought treatment is applied in the experimental plots making it unlikely that selection could differ between ambient and drought plots at this stage of the life cycle. Even if some maternal plants recruited from seed banks within each plot, the possible absence of the plants in some years, and the high possibility of gene flow from via pollen and seeds from the surrounding vegetation would impede evolutionary adaptive differences to build up between plot types. We therefore consider maternal environmental effects as the most likely cause for the measured differences between offspring originating from ambient versus drought plots, and in the following discuss our results accordingly.

Transgenerational effects

372 We found no overall difference in survival between offspring originating from maternal ambient
373 and drought plots. However, plants originating from the maternal ambient plots had overall a
374 higher above ground biomass and flower production than plants originating from the maternal
375 drought plots. Disregarding the competition treatments, plants from maternal ambient plots
376 survived drought better than plants from drought plots. These results support the *maternal*
377 *provisioning hypothesis*: offspring from the most benign maternal environment (here ambient
378 plots) produce generally better performing offspring than offspring from less benign maternal
379 environments (drought plots). There was no significant difference in mean weight of individual
380 seeds between those produced in ambient versus drought plots, albeit a trend towards lower
381 weight of seeds produced in maternal drought plots. This suggests that differences in maternal
382 provisioning of seeds between ambient and drought plots are at best small, and not a major
383 contributor to the phenotypic differences we observed between offspring from ambient and
384 drought plots. Moreover, when considering the effects of competition, our results give support to
385 the *transgenerational beneficial acclimation hypothesis*. The impact of competition on offspring
386 performance varied between maternal environments and this depended on water availability
387 during the greenhouse experiment, and on the fitness component considered (survival or
388 reproduction). Competition significantly reduced survival in offspring originating from maternal
389 ambient plots, but only in the drought treatment. Notably, this negative response to competition
390 under drought was observed for all 12 maternal families from the ambient plot (Fig. 3A drought,
391 grey lines) showing that this result was not an artefact of just few lines with a strong negative
392 response. Similarly, but in contrast to ambient families, survival in all of the ten maternal
393 families from drought plots were unaffected by competition when grown in conditions matching
394 their home maternal environment (see Fig. 3A drought, black stippled lines). For these plants,

competition even marginally increased survival (Fig 2A and 3A black lines). This suggests adaptive phenotypic response in offspring originating from drought plots when grown in their “home” maternal abiotic and biotic environment. It is possible that under drought these plants better take advantage of facilitative effects of a neighbor. Facilitative effects of neighbors that increase survival of beneficiaries are commonly documented for drought stressed environments (Callaway 2007, Michalet & Pugnaire 2016)

For reproduction, adding competition to pots containing offspring from maternal drought plots decreased their number of flowers, but only in the control water treatment (water condition different from their home maternal environment). We hypothesized that adding competition would increase the environmental stress of the focal plant growing in the most stressful environment. What defines the most stressful environment depends on whether offspring performance is mainly determined by maternal provisioning (a) or by transgenerational beneficial acclimation (b). Under hypothesis a, drought is the stressful environment, and offspring from the most benign maternal environment (here ambient plots) produce best performing offspring. Under hypothesis b, the stressful environment is the one different from the maternal one, so offspring from drought plots should perform best under competition when grown in drought compared to offspring from ambient plots, and vice versa. Competition did not exacerbate any negative impact of drought on reproduction in the offspring from either of the two maternal environments (Fig 2B; dashed lines). Offspring from ambient plots produced consistently more flowers than offspring from drought plots irrespective of competition level, suggesting different allocation strategies to reproduction depending on maternal environment. In contrast, when growing in control water treatment competition reduced flower production by 25% (Fig 2B solid black line) in offspring originating from drought plots.

418 The negative impact of competition on reproduction in offspring from drought plots when
419 growing under abiotic conditions (water availability) different from their “home” maternal
420 environments is consistent with both the maternal provisioning (plants from most benign
421 maternal environments perform best) and the beneficial acclimation hypothesis (competition
422 exacerbates stress most in the non-home maternal environment).

423 If we had focused exclusively on response to drought in the absence of competition, we would
424 not have uncovered the potential adaptive phenotypic response to the home maternal drought
425 environment (survival) or the different (reduced) allocation to reproduction when grown at a
426 moisture level different from the maternal home environment.

427 At our study site, competition from perennial grasses is pervasive (Damgaard et al 2009). Our
428 greenhouse experiment was designed to mimic the original maternal environment. We prepared
429 pots with soil (top soil and deeper soil) collected at the heathland site where maternal progenies
430 were collected, and we used a competing grass species also collected at that location. We
431 ensured that the relative difference in water availability measured in the ambient and drought
432 maternal plots was replicated in the water treatments in the greenhouse. Thus, offspring were
433 evaluated in greenhouse conditions where three crucial environmental variables (soil, competitor
434 species, and water availability) were matching those of the maternal environments.

435 Few studies examine the combined effect of the maternal biotic and abiotic environment on
436 offspring performance. Metz et al (2015) studied the effect of the maternal environment on
437 competitive ability under different watering levels in the annual herb, *Biscutella didyma*. The
438 maternal environment was considered predictable (constant across generations) with respect to a
439 high plant density (similar to our study) and unpredictable with respect to occurrence of drought.

440 Interestingly, adaptive maternal effects on a range of plant performance variables were mostly
441 found when offspring were grown with a neighbor plant (Metz et al 2015), suggesting adaptive
442 parental effect for offspring competitive ability, but not for offspring drought tolerance. A
443 notable difference between the study by Metz et al (2015) and our study is that they studied an
444 annual plant while we studied a perennial plant. Investment in survival and reproduction under
445 different environmental stressors is expected to vary between annual and perennial life history
446 strategies (Bulmer 1994; de Jong and Klinkhamer 2005; Hansen et al 2013). In annuals, fitness
447 depends on reproductive output in a single growing season, whereas in perennials, reproduction
448 in one season may be traded off for survival and future reproduction. Hence, for perennials,
449 obtaining reliable fitness related parameters is more challenging (Marshall and Uller 2007). We
450 measured plant performance over one growing season, and both reproduction and survival until
451 next year are arguably important fitness components for a short-lived perennial like *H.*
452 *umbellatum*. However, as we do not know the trade-off between reproduction and future survival
453 we hesitate to interpret phenotypic responses as adaptive.

454 We only found negative effects of competition for offspring grown under a water treatment
455 different from their maternal environment. This suggests that provided the water conditions are
456 similar to those experienced by maternal plants, offspring maintain high performance even under
457 competition. The plants originating from maternal drought environments had the lowest above
458 ground biomass. Moreover, these plants, when grown with a competitor under control water
459 conditions showed a drop in their reproduction relative to offspring from maternal ambient plots.
460 One possible explanation for this is that offspring from maternal drought plots invest more in
461 root biomass than offspring from maternal ambient plots. Indeed, a number of studies have found
462 that offspring from drought stressed maternal environments show decreased shoot/root ratio (e.g.

Sultan et al 2009; Herman et al 2012; Nosalewicz et al 2016). Alteration in assimilates to shoots and roots in favor of roots is generally considered an adaptation to drought (Nicotra et al 2010). We could not reliably estimate root biomass in the present study. Despite the fact that there were at best very modest difference in initial seed weight, and that we started the experiment with similar sized seedlings, offspring from drought plots consistently had lower above ground biomass compared to offspring from ambient plots (Fig. 4). This is consistent with the plants from maternal drought environment investing relatively more in root biomass. A higher investment in roots may give an advantage when plants are competing for water explaining the higher survival of offspring from drought plots under this environmental condition. Our results suggest that when water is not limiting, the competitive edge that plants from maternal drought environment may have relative to plants from maternal ambient environment disappears.

This study adds to a growing literature on transgenerational effects considering more ecologically realistic experimental settings by simultaneously manipulating both biotic and abiotic environmental factors. Our main finding is that the maternal environment had a strong impact on offspring fitness components with higher reproduction and biomass in offspring from the most benign maternal environment. Potentially adaptive phenotypic responses for survival were only revealed under a drought + competition condition. In that growing condition, plants from all maternal families originating from ambient water plots had reduced survival while plants from maternal families originating from maternal drought plots were not adversely affected.

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References

- Agrawal AA (2001) Transgenerational consequences of plant responses to herbivory: An adaptive maternal effect? *Am Nat* 157: 555-569
- Anderson JT, Panetta AM, Mitchell-Olds T (2012) Evolutionary and ecological responses to anthropogenic climate change. *Plant Physiol* 160: 1728-1740
- Auge GA, Leverett LD, Edwards BR, Donohue K (2017) Adjusting phenotypes via within- and across-generational plasticity. *New Phyt* 216: 343-349
- Bataillon T, Galtier N, Bernard A al (2016) A replicated climate change field experiment reveals rapid evolutionary response in an ecologically important soil invertebrate. *Glob Chang Biol* 22:2370-2379.
- Bates D, Mächler M, Bolker B et al (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67: issue 1.
- Beier C, Emmett B, Gundersen P et al (2004) Novel approaches to study climate change effects on terrestrial ecosystems at the field scale – drought and passive night time warming. *Ecosystems* 7: 583-597
- Bossdorf O, Richards CL, Pigliucci M (2008) Epigenetics for ecologists. *Ecol Lett* 11: 106-115
- Bulmer MG (1994) *Theoretical Evolutionary Ecology*. - Sunderland, MA, Sinauer Associates
- Callaway RM (2007) *Positive Interactions and Interdependence in Plant Communities*. Springer, Dordrecht, Netherlands
- Carroll SP, Hendry AP, Reznick DN et al (2007) Evolution on ecological time-scales. *Func Ecol* 21: 387-393

513 Cheptou P- O, Carrue O, Rouifed S, Cantarel A. 2008. Rapid evolution of seed dispersal in an
 514 urban environment in the weed *Crepis sancta*. Proc Natl Acad Sci USA 105: 3796–3799
 515 Damgaard C, Riis-Nielsen T, Schmidt IK (2009) Estimating plant competition coefficients and
 516 predicting community dynamics from non-destructive pin-point data: a case study with *Calluna*
 517 *vulgaris* and *Deschampsia flexuosa*. Plant Ecol 201: 687-697
 518 de Jong T, Klinkhamer P (2005) Evolutionary Ecology of Plant reproductive Strategies,
 519 Cambridge University Press, Cambridge.
 520 Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in
 521 response to a climate fluctuation. Proc Natl Acad Sci USA 104: 1278–1282
 522 Galloway LF (2005) Maternal effects provide phenotypic adaptation to local environmental
 523 conditions. New Phyt 166: 93-100
 524
 525 Galloway LF, Etterson JR (2007) Transgenerational plasticity is adaptive in the wild. Science
 526 318: 1134-1136
 527 Hansen CF, Garcia MB, Ehlers BK (2013) Water availability and population origin affect the
 528 expression of the tradeoff between reproduction and growth in *Plantago coronopus*. J Evol Biol
 529 26: 993-1002
 530 Herman JJ, Sultan SE, Horgan-Kobelski T et al (2012) Adaptive transgenerational plasticity in
 531 an annual plant: Grandparental and parental drought stress enhance performance of seedlings in
 532 dry soil. Integr Comp Biol doi:10.1093/icb/ics041

533 Herman JJ, Sultan SE (2011) Adaptive transgenerational plasticity in plants: case studies,
 534 mechanisms, and implications for natural populations. *Front Plant Sci* 2: doi:
 535 10.3389/fpls.2011.00102

536 Herman JJ, Sultan SE (2016) DNA methylation mediates genetic variation for adaptive
 537 transgenerational plasticity. *Proc. R. Soc. B.* 283: 20160988

538 Holmstrup M, Sørensen JG, Schmidt IK et al (2013) Soil microarthropods are only weakly
 539 impacted after 13 years of repeated drought treatment in wet and dry heathland soils. *Soil Biol*
 540 *Biochem* 66: 110-118

541 Huey RB, Berrigan D, Gilchrist GW et al (1999) Testing the adaptive significance of
 542 acclimation: A strong inference approach. *Am Zool* 39: 323-336

543 Marshall DJ, Uller T (2007) When is a maternal effect adaptive? *Oikos* 116: 1957-1963

544 Metz J, von Oppen J, Tielbörger K (2015) Parental environmental effects due to contrasting
 545 watering adapt competitive ability, but not drought tolerance, in offspring of a semi-arid annual
 546 Brassicaceae. *J Ecol* 103: 990-997

547 Michalet R, Pugnaire FI (2016) Facilitation in communities: underlying mechanisms, community
 548 and ecosystem implications. *Func Ecol* 30: 3-9

549 Monaghan P (2008) Early growth conditions, phenotypic development and environmental
 550 change. *Philos Trans R Soc Lond B Biol Sci* 12: 1635-1645

551 Mousseau TA, Fox CW (1998) *Maternal Effects as Adaptations*. Oxford University Press,
 552 Oxford.
 553

554 Nicotra AB, Atkin OK, Bonser SP et al (2010) Plant phenotypic plasticity in a changing climate.
 555 Trends Plant Sci 15: 684-692

556 Nosalewicz A, Siecinska J, Smiech M et al (2016) Transgenerational effects of temporal drought
 557 stress on spring barley morphology and functioning. Env Exp Bot 131: 120-127

558 Penuelas J, Prieto P, Beier C et al (2007) Response of plant species richness and primary
 559 productivity in shrublands along a north-south gradient in Europe to seven years of experimental
 560 warming and drought: reduction in primary productivity in the heat and drought year of 2003.
 561 Glob Chang Biol 13: 2563-2581

562 Roach DA, Wulff RD (1987) Maternal effects in plants. Ann Rev Ecol Syst 18: 209-235
 563

564 Rottstock T, Kummer V, Fischer M et al (2017) Rapid transgenerational effects in *Knautia*
 565 *arvensis* in response to plant community diversity. J Ecol 105: 714-725

566 Shaw RG, Etterson JR (2012) Rapid climate change and the rate of adaptation: insight from
 567 experimental quantitative genetics. New Phyt 195: 752-765

568 Sowerby A, Emmett BA, Tietema A et al (2008) Contrasting effects of repeated summer drought
 569 on soil carbon efflux in hydric and mesic heathland soils. Global Change Biol 14: 2388-2404

570 Stearns S (1992) The Evolution of Life Histories. Oxford University Press, Oxford.
 571

572 Sultan SE, Barton K, Wilczek AM (2009) Contrasting patterns of transgenerational plasticity in
 573 ecologically distinct congeners. Ecology 90: 1831-1839

574 terHorst CP, Lau JA (2011) Direct and indirect transgenerational effects alter plant-herbivore
575 interactions. *Evol Ecol*. DOI 10.1007/s10682-012-9560-8

576 Violle C, Castro H, Richarte J et al (2009) Intraspecific seed trait variations and competition:
577 passive or adaptive response? *Func Ecol* 23: 612-620

578 Walther J, Harter DEV, Beierkuhnlein C et al (2016) Transgenerational effects of extreme
579 weather: perennial plant offspring show modified germination, growth and stoichiometry. *J Ecol*
580 104: 1032-1040

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Legends to figures

Figure 1. Overview of the experimental plots at the Mols site showing the open sided scaffolding and close proximity of plots.

Figure 2. Interaction plot of survival (A) and flower number (B) of offspring grown in control water treatment (solid lines) and drought treatment (dashed lines) with and without a competitor. The grand mean of offspring originating from maternal ambient and drought plots is computed for each growing condition.

Figure 3. Reaction norm of individual maternal families growing under two different greenhouse water treatments: Control and Drought. Plants were growing either alone (no competition) or in competition with a perennial grass. 3A: Survival, 3B: Flower number.

Figure 4. Dry weight of total aboveground biomass (vegetative plus reproductive tissue) of offspring originating from maternal ambient and drought environments grown under two different greenhouse water treatments (Control and Drought) and either in competition with a perennial grass or alone.

606 **Table legends**

607

608 Table 1. Mean annual soil temperature (at 5 cm depth), precipitation and soil moisture content at
609 the end of the applied drought period in control (ambient) and drought plots. Means ($\pm$ SE) are
610 based on 13 years measurements from (2000-2012).

611 Table 2. Summary of linear mixed models analysis

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616

617 Table 1

	Ambient	Drought
Mean yearly soil temperature (°C)	8.9 ($\pm$ 0.13)	8.9 ($\pm$ 0.12)
Mean annual precipitation (mm)	693 ($\pm$ 27)	539 ($\pm$ 21.4)
Final soil moisture (m ³ m ⁻³) after drought period	0.123 ($\pm$ 0.0078)	0.067 ($\pm$ 0.0036)

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Figure 1

Figure 2

Figure 3

Figure 4

Table 2: Summary of linear mixed models fit for offspring phenotypic responses

	Flowering date			Log(Flower Number)		
	MS	F	P-value	MS	F	P-value
Fixed effects						
Competition	1.562	0.142	0.707	0.014	0.308	0.579
Water Treatment	19.453	1.767	0.260	0.290	6.509	0.065 ‘
Maternal Origin	4.900	0.445	0.512	0.191	4.292	0.050 *
Competition*Water Trt	0.554	0.050	0.823	0.163	3.647	0.057 ‘
Competition*Maternal Origin	0.189	0.017	0.896	0.001	0.030	0.864
Water Trt*Maternal Origin	9.450	0.858	0.355	0.022	0.482	0.488
Competition*Water Trt*Mat. Origin	20.943	1.902	0.169	0.002	0.054	0.817
Random effects	Var (%) ^a	G	P-value	Var (%)	G	P-value
Family (Maternal origin)	1.79 (13.9)	23.9	<0.0001 ***	0.0084 (15.2)	28.8	<0.0001 ***

	Vegetative Biomass			Total Biomass		
	MS	F	P-value	MS	F	P-value
Fixed effects						
Competition	1.535	0.099	0.753	1.561	0.068	0.794
Water Treatment	222.900	14.400	0.023*	222.040	9.741	0.039 *
Maternal Origin	55.269	3.570	0.072 ‘	93.881	4.119	0.055 ‘
Competition*Water Trt	0.220	0.014	0.905	0.068	0.003	0.957
Competition*Maternal Origin	0.824	0.053	0.818	3.320	0.146	0.703
Water Trt*Maternal Origin	2.239	0.145	0.704	2.296	0.101	0.751
Competition*Water Trt*Mat. Origin	12.344	0.797	0.373	19.114	0.839	0.360
Random effects	Var (%)	G	P-value	Var (%)	G	P-value
Family (Maternal origin)	3.18 (16.9)	30.9	<0.0001 ***	4.88 (17.3)	33.0	<0.0001 ***

Notes

a: for the Family random effect, we report the variance estimate as well as the percentage of variance explained by the factor. The variance attributable to cell random effects were invariably small and not significant and not reported here.

Statistical significance is indicated with ‘ for P-values <0.1, * for P-values < 0.05, *** for P-values < 0.0001