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RESEARCH ARTICLE

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Climate-driven evolutionary change in reproductive and early-acting life-history traits in the perennial grass *Festuca ovina*

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Abstract

1. Reproductive and early-acting life-history traits are likely to be particularly important determinants of plant fitness under a changing climate. There have, however, been few robust tests of the evolution of these traits under chronic climate change in natural ecosystems. Such studies are urgently needed, to evaluate the contribution of evolutionary change to population persistence.
2. Here, we examine climate-driven evolutionary change in reproductive and early-acting plant life-history traits in the long-lived perennial plant, *Festuca ovina*.
3. We collected established plants of *F. ovina* from species-rich calcareous grassland at the Buxton Climate Change Impacts Laboratory (BCCIL), after 17 years of in situ experimental drought treatment.
4. P1 plants collected from drought-treated and control (ambient climate) plots at BCCIL were used to create an open-pollinated F1 progeny array, which was subsequently validated using microsatellite markers to establish a robust bi-parental pedigree. We measured the timing of germination and seed mass in the F1 progeny, the P1 paternal contribution to F1 offspring (paternal reproductive success), and assessed the effects of flowering time on the mating system.
5. F1 seed with ancestry in drought-treated plots at BCCIL germinated significantly later than seed derived from individuals from control plots. P1 plants from the drought treatment flowered significantly earlier than those from the control plots in summer 2012, but not in 2013. Male reproductive success was also lower in P1 plants collected from drought plots than those from control plots. Furthermore, our pedigree revealed that mating among parents of the F1 progeny had been assortative with respect to flowering time.
6. *Synthesis*. Our study shows that chronic drought treatment at Buxton Climate Change Impacts Laboratory has driven rapid evolutionary change in reproductive and early-acting life-history traits in *Festuca ovina*, and suggests that evolutionary differentiation may be reinforced through changes in flowering time that reduce the potential for gene flow.

KEYWORDS

assortative mating, calcareous grassland, climate change, drought, evolution, *Festuca ovina*, germination, male reproductive success

1 | INTRODUCTION

Reproductive and early-acting plant traits defining viability are expected to be pivotal determinants of fitness under a changing climate (Donohue, Casas, Burghardt, Kovach, & Willis, 2010; Etterson & Mazer, 2016). Together, these traits underpin demography and survival in the next generation, and their evolution is highly likely to influence population growth, and may alter population persistence (Etterson & Mazer, 2016). Furthermore, reproductive traits are also key determinants of both the plant mating system—the pattern of mating among plants within a population—and gene flow between populations (Barrett & Harder, 2017; McNeilly & Antonovics, 1968; Snaydon & Davies, 1976). Hence, their evolution may constrain or reinforce the evolution of other plant phenotypes, and influence levels of inbreeding and the maintenance of genetic variation (Eckert et al., 2010). Understanding how the climate shapes these traits, therefore, is critical to understanding how, and whether plant populations will evolve in response to, or be buffered from the effects of climate change (Hoffmann & Sgro, 2011).

A number of studies now indicate the potential for plant populations to evolve in response to climatic factors, and have identified some of the traits that respond to selection (reviewed in Franks, Weber, & Aitken, 2014). For example, evolutionary changes in flowering time associated with climatic variation have been demonstrated in *Betula pubescens* and *Betua pendula* (Billington & Pelham, 1991), *Brassica rapa* (Franks, Sim, & Weis, 2007), and *Triticum dicoccoides* and *Hordeum spontaneum* (Nevo et al., 2012). Climate-associated evolutionary changes in reproductive output have also been detected in *Polygonum cespitosum* (Sultan, Horgan-Kobelski, Nichols, Riggs, & Waples, 2013). However, we still know little about the effects of chronic climate change in the field, and there have been very few experimental studies of climate-driven genetic or evolutionary responses within natural plant populations (Avolio, Beaulieu, & Smith, 2013; Jump et al., 2008; Ravenscroft, Fridley, & Grime, 2014; Ravenscroft, Whitlock, & Fridley, 2015). Here, we investigate evolutionary responses to chronic drought in plant reproductive traits, the plant mating system and germination traits.

In hermaphroditic plants, reproductive fitness comprises a female component (the number of offspring derived from seed) and a male component (offspring sired by pollen; Primack & Kang, 1989). Female reproductive potential (seed output) can be measured easily and directly, and responds to abiotic conditions, including CO_2 levels, temperature stress and water deficit (Guilioni, Wéry, & Lecoeur, 2003; Prasad, Staggenborg, & Ristic, 2008; Wang, Taub, & Jablonski, 2015). This trait can also evolve rapidly (Sultan et al., 2013), over fine spatial scales (Antonovics & Bradshaw, 1970; Snaydon & Davies, 1972, 1976), and in response to microclimatic variation (Gonzalo-Turpin & Hazard, 2009).

Male reproductive success, on the other hand, is challenging to quantify directly in natural populations, and consequently, few studies have investigated the conditions that influence it (Austen & Weis, 2016; Bertin, 1988). The available evidence suggests that male fitness in plants can be affected by temperature (Jóhannsson & Stephenson, 1998; Pasonen, Pulkkinen, & Kärkkäinen, 2002), CO_2 concentration (Marshall et al., 2010) and nutrient availability (Lau & Stephenson, 1993, 1994; Poulton, Koide, & Stephenson, 2001; Young & Stanton, 1990). These studies establish the potential for climate change to alter male reproductive success, modifying plant mating systems and the functional sex of hermaphrodite individuals (Conner, Rush, Kercher, & Jennetten, 1996; Devlin & Ellstrand, 1990; Ennos & Dodson, 1987; Marshall et al., 2010; Snow & Lewis, 1993). However, the impacts of climate change on male reproductive success under realistic field conditions have not been quantified.

Reproductive phenology has advanced in line with the warming climate in many species and ecosystems (Cook, Wolkovich, & Parmesan, 2012; Fitter & Fitter, 2002; Menzel et al., 2006; Parmesan & Hanley, 2015). Flowering time shows high levels of phenotypic plasticity (Franks et al., 2014) and can also evolve rapidly in response to altered environmental conditions such as drought (Anderson, Inouye, McKinney, Colautti, & Mitchell-Olds, 2012; Franks et al., 2007; Nevo et al., 2012). Reproductive phenology is likely to be a key target of selection under climate change and, because of its fundamental role in mediating the plant mating system and gene flow, will be an important trait determining subsequent evolutionary change (Anderson et al., 2012; Mungu ía-Rosas, Ollerton, Parra-Tabla, & De-Nova, 2011).

The environmental cues controlling seed dormancy and germination are being modified by changing temperature, precipitation and light regimes (Walck, Hidayati, Dixon, Thompson, & Poschlod, 2011). Germination and early establishment traits have significant heritable variation, are under strong selection, and often contribute strongly to local adaptation (reviewed by Baskin & Baskin, 2014; Donohue et al., 2010). Thus, we can expect these traits to evolve during climate change (Donohue et al., 2010). Population-level changes in germination phenology may also affect species' geographical distributions and community structure, since this trait forms a key part of a plant's adaptive life-history strategy and habitat preference (Baskin & Baskin, 2014; Donohue et al., 2010; Fenner & Thompson, 2005).

The Buxton Climate Change Impacts Laboratory (BCCIL) provides an excellent experimental platform with which to investigate evolutionary responses to climate change. At this site, a natural species-rich grassland has been subjected to a range of experimental climate change treatments since 1993. During the first 13 years of treatments, this grassland has shown resistance to simulated climate change, with community composition remaining relatively stable through time (Grime et al., 2000, 2008). However, at fine spatial scales there has been significant reorganization of community structure, associated with centimetre-scale edaphic variation that locally modifies the effects of the climate treatments (Fridley, Grime, Askew, Moser, & Stevens, 2011). Subsequently, community change has been characterized by gradual change in species abundance, underpinned by differences in species' functional traits (Fridley, Lynn, Grime, & Askew, 2016). Evolution may be one of the mechanisms limiting climate-driven change in grassland community structure at BCCIL (Grime et al., 2008). Recently, we have used molecular markers to document climate-driven genetic changes at BCCIL, within

populations of *Festuca ovina* and *Plantago lanceolata* (Ravenscroft et al., 2015). Phenotypic differentiation among climate treatments has also been described for *P. lanceolata* (Ravenscroft et al., 2014), but the processes underpinning this differentiation were not resolved. Thus, while there is clear evidence for genetic and phenotypic change within plant populations at BCCIL, evolutionary changes in phenotype have not yet been documented.

Here, we investigate evolution in response to long-term selection under drought treatment at BCCIL, within a population of the perennial grass *F. ovina*. We describe the construction and molecular validation of an open-pollinated F1 progeny array from field-collected parental plants, to facilitate analysis of heritable drought-induced evolution. We combine this resource with data from common garden experiments to ask whether long-term drought treatment has driven evolutionary changes in key plant traits associated with reproduction, germination and the plant mating system, and to document the nature of drought-induced phenotypic change.

2 | MATERIALS AND METHODS

2.1 | Study site and study species

Climate treatments at BCCIL have been applied annually to 3 × 3 m grassland plots, beginning in 1993. Each treatment is replicated five times in a randomized block design (for full details see Grime et al., 2000; Grime et al., 2008). In this study, we focussed on the drought treatment and control plots, because this treatment has driven the largest changes in species abundance (Fridley et al., 2011), and therefore is likely to generate the greatest selection pressures and potential for evolution. The drought treatment is imposed using rain shelters during July and August, and leads to a significant reduction in the surface soil water potential by the end of the treatment ($\psi_{drought}$ = −1,100 kPa; $\psi_{control}$ = −20 kPa; (Fridley et al., 2011).

Festuca ovina (L.) is a perennial, wind pollinated grass with hermaphroditic, self-incompatible flowers (Ghatnekar, 1999; Stace, 2010; Weilbull, Ghatnekar, & Bengtsson, 1991). It is the most abundant grass at BCCIL and has increased in abundance in the drought plots relative to control plots (Fridley et al., 2011). Non-reproductive vegetative biomass varies significantly among *F. ovina* individuals, indicating that some plants are capable of clonal growth (Bilton, Whitlock, Grime, Marion, & Pakeman, 2010). However, a genetic study of the *F. ovina* population at BCCIL that sampled 360 plants (12 from each plot), found that all individuals of *F. ovina* were genetically distinct individuals (Ravenscroft et al., 2015). This finding indicates that recruitment from seed is an important component of reproduction in this population, and that clonal growth may have only limited importance in shaping responses to the climate treatments. Furthermore, sexually reproducing *F. ovina* plants have been observed in every plot at BCCIL (S. Buckland, unpublished data), and can be observed in every year. Although *F. ovina* can be a long-lived perennial, our experience of growing this species in the common garden indicates that some individuals are short lived perennials (lifespan <5 years). Thus, the climate treatments at BCCIL are likely to have imposed selection on *F. ovina* over multiple generations. Our previous genetic analysis of the *F. ovina* population at BCCIL has also documented genetic differentiation among climate treatments that is synonymous with an evolutionary response (Ravenscroft et al., 2015).

2.2 | Collection and propagation of *F. ovina*

In July 2010, individuals of *F. ovina* and three other species were collected from the drought and control plots at BCCIL after 17 years of climate manipulation. Results for species other than *F. ovina* will be presented elsewhere. Thirty *F. ovina* individuals were collected, using a spatially stratified, randomized sampling design, from each of these climate environments (drought and control; six individuals per plot, per environment). Physically connected bunches of 4–8 tillers were recovered from each sampled plant and potted in cell trays in John Innes No. 1 compost. These clonal lines were subsequently allowed to establish in 3 L pots containing a 3:1 mix of John Innes No. 1 potting compost and medium grade Perlite at Ness Botanic Gardens, University of Liverpool, UK (full details are provided in Appendix S1). They were maintained by biomass clipping 25 mm above the soil surface in September to mimic grazing and to promote clonal growth, and by seed head removal during July 2011, to prevent self-seeding. The clonal lines received natural rainfall, supplemented with a pumped ground-water supply in dry periods. Hereafter, these 59 field-collected clonal lines (one individual died following collection) are referred to as the parental or P1 plants.

2.3 | Creation of an F1 progeny array

We created an F1 progeny array to allow investigation of heritable changes in plant phenotypes induced by experimental climate change at BCCIL. By June 2012, the parental (P1) plants had formed large tussocks comprising hundreds of tillers, and all but one of the clones flowered in this year, producing between 2 and 104 flowering tillers (the number of flowering tillers were recorded). We allowed the plants to cross-fertilize naturally by wind pollination during June 2012. During the flowering period, the relative spatial location of the pots was altered daily by moving a subset of pots according to a systematic schedule (Appendix S1). We did this to minimize the effects of spatial location on F1 offspring paternity. Since *F. ovina* is an outcrossing hermaphrodite plant, each clonal line was expected to function as both a maternal parent (producing seed) and a paternal parent (through pollen production), with mating occurring only among distinct plant genotypes.

During July 2012, we collected seed from each of the P1 parent plants to create F1 offspring. From these bulk collections of seeds, 16 seeds from each parent clone were selected at random and weighed individually, leading to a balanced design with respect to the maternity of the selected seeds. These 16 seeds were placed on filter paper within a 90 mm Petri dish with 1.4 ml of Milli-Q ultra-high pure

water (18.2 MΩ·cm). Seed dishes were placed in a fridge for 24 hr before being moved to an indoor space at room temperature with natural lighting, with randomized location with respect to the maternal plant's origin at BCCIL. Each dish was watered twice a week with 1 ml of Milli-Q ultra-high pure water. Seeds were checked daily to score germination, which was defined as the emergence of the coleoptile. Recording started on 7 August 2012 and final observations were taken on 4 October 2012. Seeds not germinated by the final date were recorded as having failed to germinate.

Eight seedlings were selected randomly for each parent clonal line from those that germinated, and planted into seed trays (24 cell trays, each pot 5 × 5 × 5 cm) containing a 1:2:1 mix of natural rendzina soil, John Innes No. 1 compost and perlite. These 472 F1 individuals (F1 offspring plants) were allowed to establish outdoors at Ness Botanic Gardens, and were managed as described for the P1 plants.

2.4 | Genomic DNA extraction

We collected and dried leaf tissue from all of the P1 and F1 individuals, and extracted genomic DNA from these samples (following Whitlock, Hipperson, Mannarelli, & Burke, 2008). A replicate collection of leaf tissue was also taken from 37 randomly selected P1 clones, to allow estimation of genotyping error.

2.5 | Microsatellite marker development and genotyping

Microsatellite markers for *F. ovina* were developed from transcriptome sequence data, in order to determine the full pedigree of the F1 progeny array (Appendix S2). In brief, after identification of sequences containing microsatellite repeat motifs, 48 candidate microsatellite loci were screened, and of these, nine loci were retained that showed high levels of polymorphism and consistent amplification (Table S1). We used these loci to genotype the P1 parent and F1 offspring plants, and the replicate P1 tissue samples. PCR amplifications were carried out in two multiplexes, using the Qiagen Multiplex PCR Kit (Qiagen; thermocycling conditions are given in Appendix S2). Microsatellite loci were scored using semi-automated methods within the software GeneMapper Version 3 (Applied Biosystems). We made up to four allele calls per locus in each individual, since the BCCIL population of *F. ovina* is tetraploid. All allele calls were checked manually, and where these were ambiguous they were removed (Appendix S3). The final dataset consisted of microsatellite genotypes for 553 individuals (457 F1 offspring and 59 P1 parents, including replicate genotypes for 37 of the parent individuals).

2.6 | Common garden experiment

We used a common garden experiment to assess variation and climate-induced differentiation in flowering time among the parental P1 clonal lines. In September 2011, four replicates of each of the P1 individuals were planted in pots containing natural rendzina soil and grown under standardized conditions, at each of two soil depths (Appendix S7). Flowering time was measured in summer 2012 and summer 2013 by monitoring inflorescences twice a week during flowering. The day of anthesis was recorded for each inflorescence. Inflorescences recorded as having reached anthesis were removed immediately.

2.7 | Statistical analyses

All analyses were carried out in R version 2.15.1+ (R Development Core Team, 2008). We assessed the power of our microsatellite markers to discriminate among related plant individuals by calculating the total multi-locus $P_{(ID)}$ (following Waits, Luikart, & Taberlet, 2001; Appendix S4). The clonal uniqueness of the P1 plants was assessed using the *assignclones* function using the *meandistance.matrix*2 function (R package polysat), using Bruvo genetic distances, with selfing rate set to 0.1, and with the threshold level set at 0.2 (this threshold indicates the maximum genetic distance between two individuals that will be placed in the same clonal group; (Clark, 2014; Clark & Jasieniuk, 2011).

The pedigree of the F1 progeny array was determined using full probability Bayesian parentage analysis using the R package masterbayes (Hadfield, Richardson, & Burke, 2006). Since masterbayes does not handle tetraploid genotypes, the microsatellite genotypes for each individual were converted to binary (presence-absence) genotypes using the *genambig.to.genbinary* function in polysat (Clark & Jasieniuk, 2011). These binary genotypes were subsequently treated as dominant markers. The *pdataped* argument of masterbayes was used to constrain the pedigree model, such that only P1 individuals could act as parents, with no selfing, and to specify the maternal parent of each F1 offspring. No other informative priors were specified and the model was allowed to estimate error rates (Hadfield et al., 2006). The parentage model was run for 1,500,000 iterations, with a thinning interval of 700 and a burn in of 800,000 iterations. We stored the posterior probability distribution for all parentage inferences with a confidence level of at least 50%. Since the samples from the MCMC chain estimating genotyping error rate E1 showed autocorrelation of 0.323, we carried out a sensitivity analysis to test its influence on pedigree estimation (Appendix S5). Re-running the pedigree under a variety of scenarios resulted in a minimum 78.3% agreement with the final pedigree used. The most probable paternity assignments were used to establish the pedigree of the F1 progeny array (Table 1), defining the *ancestral climatic environment* at BCCIL of each F1 plant (one of either drought, hybrid [control–drought, drought–control], or control ancestries).

Climate-driven differentiation in P1 male reproductive success—the number of offspring sired by each parent via pollen—and flowering tiller number were assessed using generalized linear models (GLM), via the R package mcmcglmm (Hadfield, 2010; full model specifications are given in Appendix S6). We also tested whether the male

TABLE 1 Summary of F1 climate ancestries defined by paternity analysis applied to the F1 progeny array

Maternal environment		Paternal environment	Offspring climatic ancestry	*n*
Control	×	Control	Control	135
Control	×	Drought	Hybrid	208
Drought	×	Control		
Drought	×	Drought	Drought	88

Note: Sample sizes show frequencies of individuals in the F1 progeny array. The columns labelled maternal and paternal environment show sampling environments for parent plants at BCCIL

Abbreviation: BCCIL, Buxton Climate Change Impacts Laboratory.

reproductive success was related to the number of flowering tillers that parent plants produced (the latter is a crude measure of total effort in pollen production).

We used a Pearson's chi-squared homogeneity test to determine whether mating was assortative with respect to the climate treatment from which parent plants had been sampled at BCCIL. A permutation test was used to determine whether mating was assortative with respect to parent plant flowering time, using data from the common garden experiment, as follows. First, an average flowering time was defined for each P1 parent clone as the mean number of days to anthesis for the earliest flowering inflorescence across replicate plants in the common garden. To conduct the permutation test we used these data to construct a matrix of the expected dissimilarity in flowering time between all possible pairs of P1 parent plants (excluding self pairs; n = 1,653 possible parent crosses). A single parent plant (and a mating event associated with its single progeny) was excluded from this analysis because it had no flowering time information. We observed 434 mating events among P1 parent individuals in our F1 progeny array. Therefore, to set up a null distribution for flowering time dissimilarity, we drew 99,999 random samples of size 434 from the complete matrix of flowering time dissimilarity. For each draw, we calculated the mean flowering time dissimilarity, as a summary statistic for flowering time differences between mating plants. The distribution of these summary statistics represents a null distribution for flowering time dissimilarity, defining the expectation if mating were at random. The observed value of mean flowering time dissimilarity was also added to this set, to give 100,000 values. The observed mean value of the flowering time differences was then compared to the null distribution. Assortative mating by flowering time was also assessed as a simple correlation in the clonal mean flowering time between P1 parent clones that were identified as having mated by the pedigree (Wright, 1921).

We used linear mixed modelling (LMM) to test for differences in P1 flowering time between climate treatments, and to estimate the clonal broad-sense heritability of flowering time (Lynch & Walsh, 1998). The timing of anthesis of individual inflorescences was used as the response variable. Treatment and soil depth were fitted as fixed effects and P1 clone identity and pot identity were fitted as random effects (the latter random effects to account for correlated pot effects on flowering time). Other experimental blocking factors were fitted either as centred fixed effects or as random effects (Appendix S7). Genetic repeatability of P1 plant flowering time between years was tested using simple regression applied to clonal mean flowering time data.

We used generalized linear mixed modelling (GLMM) to test whether seed mass and climatic ancestry influenced the extent or timing of F1 seed germination. First, we used GLMM to assess whether ancestral climatic environment at BCCIL or seed mass predicted whether or not a seed germinated. This analysis was conducted on all the seeds that were initially planted, and therefore paternal information was not available for more than half of the sample, hence no paternal information was fitted in this model (n = 914). Seed mass and maternal ancestral climate at BCCIL were fitted as fixed effects. Maternal (P1) clone identity was fitted as a random effect.

For the F1 seed with complete pedigree information (n = 431), we used GLMM to investigate whether seed mass was predicted by climatic ancestry. Parental climate ancestry was fitted as a fixed effect with three levels (Table 1; *control*, *hybrid* and *drought ancestry*). Maternal and paternal clone identity were fitted as random effects.

Finally, we used survival analysis to examine the association between germination timing and climatic ancestry F1 progeny plants, using the R package SURVIVAL (Therneau & Grambsch, 2000). Survival analysis is used to analyse the time to an event, often death, or component failure. Here, we modelled the time delay, or latency, from seed imbibition to germination. We fitted parametric survival regression models containing both parental ancestry and seed mass as explanatory variables, using the Weibull distribution. Nested (simplified) models were fitted by removing predictor variables, and the best fitting model was assessed by comparison of Akaike Information Criterion values.

3 | RESULTS

3.1 | Microsatellite genotyping and paternity analysis

We used 9 microsatellite markers to genotype the P1 parent and F1 offspring plants. 518 individuals (93.7% of the total) were successfully genotyped at 8 or more of the 9 microsatellite loci (comprising 55 of 59 parents, 93.2%; 36 of the 37 replicated samples, 97.3%; and 427 of the 457 offspring, 93.4%). The total number of alleles per locus across individuals ranged from 6 to 20 (Table 2), and the average allelic richness per individual across loci was 18.8. The total multi-locus $P_{(ID)sib,}$ a measure of the power of microsatellite markers to discriminate among related individuals, was 2.861×10^{-5}. This means that the probability of two full sibs plants having the same microsatellite genotype would be extremely low with this marker set. Each P1 parental clone sampled from BCCIL was distinguished by the markers as genetically unique. Paternity assignments with a

TABLE 2 *Festuca ovina* microsatellite marker and population genetics summary statistics

Marker	Total no. alleles	No. of individuals scored
T_02	11	546
T_06	19	527
T_19	20	505
T_23	16	530
T_25	10	547
T_26	6	504
T_28	16	550
T_35	9	462
T_42	17	474

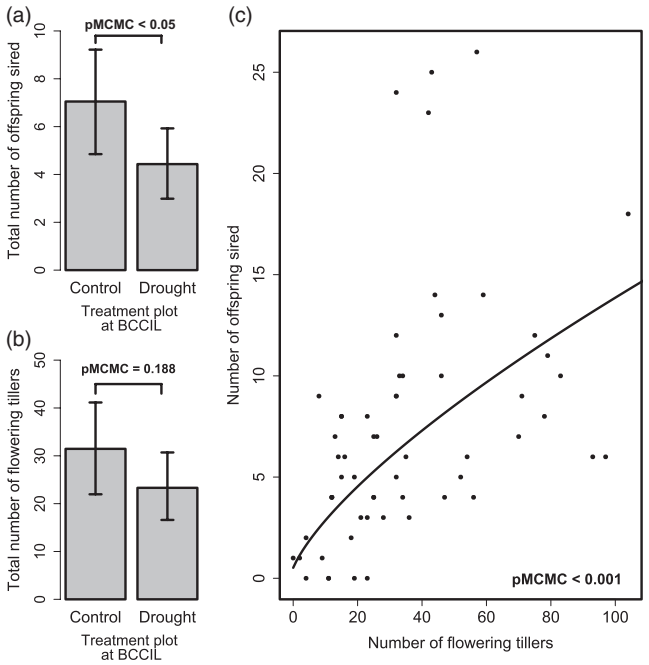

FIGURE 1 Evolutionary differentiation in reproductive success between *Festuca ovina* populations from different climate treatments at Buxton Climate Change Impacts Laboratory (BCCIL). (a) Male reproductive success, measured as the number of offspring sired per parent plant. (b) Female reproductive success, measured as the number of flowering tillers per parent plant. (c) Relationship between male and female reproductive success, measured as above. The curve represents the predicted relationship between male and female reproductive success, estimated from the MCMCGLMM model output. Error bars represent 95% credible intervals

probability of 1 were made for 249 out of 457 of the *F. ovina* F1 offspring (54.5% of the total). 397 (86.9%) individuals had paternity assignments with a probability of greater than 0.8, and 435 (95.2%) of individuals had paternity assigned with a probability of at least 0.5 (i.e. with a most likely father). A visual representation of the pedigree and pedigree summary statistics are provided in Appendix S8, Table S2, Figure S1.

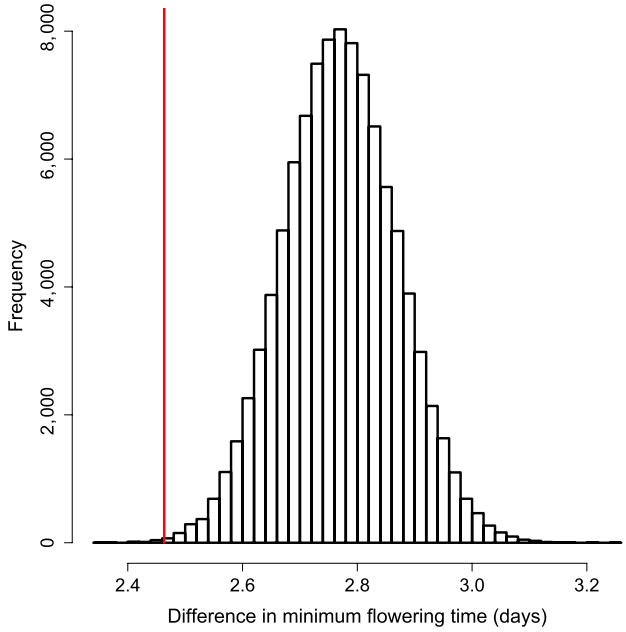

FIGURE 2 A histogram of the null distribution of mean differences in flowering time following 99,999 samples of 434 from a 1,653 matrix containing all of the possible differences in flowering time. The red line shows the location of the mean difference in flowering time that we observe in the P1 parent plants [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Genetic differentiation in reproductive and mating system traits

Our F1 progeny array and pedigree allowed us to retrospectively assess alteration of the plant mating system by chronic drought treatment at BCCIL. Since mating in P1 parent plants from BCCIL was obseved in the common garden, any shifts in the mating system reflect broad-sense genetic changes between P1 *F. ovina* populations occupying different climate treatments at BCCIL. The progeny array was created via natural wind pollination, leading to a balance in the representation of P1 materal plants, but with potential variation in P1 male reproductive success. Only five out of the 59 parent plants failed to successfully sire F1 offspring plants. The variation in P1 male reproductive success (i.e. in the number of offspring sired), ranged from 0 to 26. The number of offspring sired differed significantly between P1 ancestral climate treatments at BCCIL (Poisson GLMM; *pMCMC* = .048; *n* = 59). Plants from drought plots at BCCIL sired 37.1% fewer offspring than those from control plots, (mean offspring sired, control = 7.0; drought = 4.4; Figure 1a). P1 plants from the drought treatment at BCCIL also produced, on average, fewer flowering tillers than plants from the control treatment, although this difference was not significant (Figure 1b; Gaussian GLMM; *pMCMC* = .188; *n* = 59). The realized paternal reproductive success of P1 parents was significantly positively correlated with the number of flowering tillers that the plants had produced in the common garden, an indicator of the plants' potential for seed and pollen production (Figure 1c; Poisson GLMM; *pMCMC* < .001; *n* = 59).

The range in clonal mean flowering time for the 58 P1 plants was 17.3 days in 2012 and 17.0 days in 2013. P1 plants from the drought treatment flowered on average 1.7 days earlier than control plants in 2012 (Gaussian LMM; *pMCMC* = .038; *n* = 809 inflorescences) and 0.29 days later than control plants in 2013 (Gaussian LMM; *pMCMC* = .586; *n* = 6,672 inflorescences). Flowering time was heritable in the broad sense in both 2012 (H^2 = 41.6%) and 2013 (H^2 = 40.4%), and P1 plant flowering time was weakly repeatable between years (r^2 = .286).

The reconstruction of the pedigree, which resolved the biparental climatic ancestry of F1 plants, enabled us to assess patterns of mating among P1 parents in relation to climate treatment of origin at BCCIL, and P1 plant flowering time. Our pedigree did not provide evidence that mating among P1 parent plants had been assortative with respect to their origin in different climate treatments at BCCIL (test of independence; χ^2 = 0.642; *df* = 1; *p* = .422). Plants from the same treatment were not more likely to mate with each other in the common garden. However, mating among P1 parent plants was assortative with respect to flowering time. Plants with more similar flowering times were significantly more likely to mate with each other, than expected had mating occurred at random (two-tailed permutation test; *p* = .002; Figure 2). Under random mating we would have expected to see a mean difference in flowering time of 2.77 days. We observed a mean difference in flowering time of 2.46 days. The genetic correlation in mean flowering time between mating P1 clones was 0.157 (*p* = .001).

3.3 | Evolutionary change in germination traits

In total, 92.5% of the seeds germinated, producing F1 plants (average seed mass = 0.745 mg). The probability of germination was significantly predicted by seed mass (binary GLMM; *pMCMC* < .001; *n* = 914), with heavier seeds more likely to germinate (average mass of germinating seeds = 0.770 mg, *n* = 846; average mass of seeds failing to germinate = 0.424 mg; *n* = 68; Figure S2).

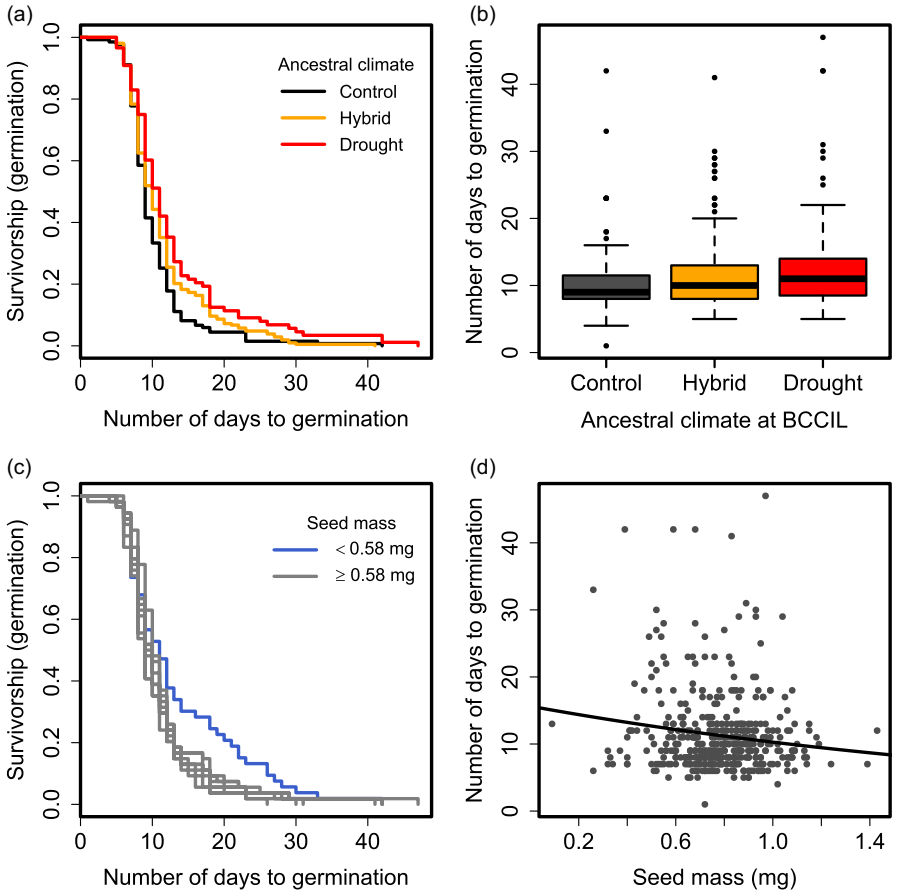

FIGURE 3 Responses of germination latency to climate ancestry and seed mass (a) Kaplan–Meier plot showing predicted germination schedules for seeds with ancestry in different climate treatments at Buxton Climate Change Impacts Laboratory (BCCIL). (b) Boxplot summarizing germination latency data by parental ancestral climate. The box represents the first and third quartile, the whiskers extend to ±1.5 × interquartile range, and points lying outside the range of the whiskers represent outliers. (c) Kaplan–Meier plot showing predicted germination schedules for seeds in different mass categories. The seed masses were grouped into eight categories with approximately equal sample sizes: 0.09–0.57 mg, *n* = 53; 0.58–0.67 mg, *n* = 54; 0.67–0.72 mg, *n* = 54; 0.72–0.78 mg, *n* = 54; 0.78–0.84 mg, *n* = 54; 0.84–0.89 mg, *n* = 54; 0.90–0.97 mg, *n* = 54; 0.97–1.43 mg, *n* = 54. (d) The lag to germination by seed mass. The trend line is the relationship between seed mass and germination latency estimated from the SURVIVAL model. Each data point represents the germination time and seed mass for a single seed [Colour figure can be viewed at wileyonlinelibrary.com]

For seeds that germinated, seed mass and climate ancestry both had significant effects on the lag to germination (parametric survival regression model, Weibull distribution; seed mass effect, $p < .001$; climate effect, $p < .001$; Figure 3). The mean lag to germination in seeds of pure drought ancestry was 2.8 days, 27.5% greater than for seeds of pure control ancestry (Figure 3a,b). Seeds of hybrid climate ancestry showed an intermediate germination latency (Figure 3a,b). The ancestral climate of F1 seeds also affected the shape of the germination latency curve, with the offspring of pure control ancestry having a smaller range in lag to germination than offspring descended from drought-treated plots (Figure 3a,b). Seed mass was negatively associated with the lag to germination, with heavier seeds tending to germinate earlier (Figure 3d). This effect was driven in large part by the late germination of seeds with the smallest masses (Figure 3c). However, there was no significant difference in seed mass between F1 seeds with pure control ancestry (both parents originating from control plots) and those of pure drought ancestry (mixed effect model; *pMCMC* = .652; $n = 431$).

4 | DISCUSSION

Here, we asked whether climate change, in the form of chronic drought stress, is capable of driving rapid evolutionary changes in reproductive traits, in the plant mating system and in seed germination traits. We used a long-term drought manipulation, applied to an intact grassland ecosystem at BCCIL to address this question. We found that plants from the long-term drought treatment have reduced potential for male reproductive success, related to their total investment in sexual reprodution (flower production). Furthermore, we have shown that mating in the *F. ovina* population from BCCIL is assortative with respect to flowering time. In one of the two years that we recorded plant flowering time, drought plants flowered significantly earlier than control plants. Therefore, periodical assortative mating by flowering time may drive the partial reproductive isolation of populations from different climate treatments at BCCIL, and enhance climate-driven evolutionary change. Finally, we detected differences in the germination time between F1 plants with ancestry in different climate treatments at BCCIL, with greater delays to germination occurring in plants whose parents both originated from the drought treatment at BCCIL. Taken together, our results reveal rapid (<17-year), climate-driven evolutionary changes in critical reproductive and early-acting life-history traits in *F. ovina*.

4.1 | The case for climate-driven evolution

The demonstration of a climate-driven evolutionary responses typically requires evidence that (a) there is heritable genetic variation in the trait of interest, (b) the trait is under climatic selection, and (c) there is a difference in the value of the trait as a result of changes in climate (Merilä & Hendry, 2014). The phenotypic differentiation in flowering time, male reproductive success and flowering tiller number that we have documented was observed in plants sampled as established individuals from the field and then grown in a common environment for three years. Both differences in flowering time and inflorescence number are heritable in the broad sense. Our recent data also show that the number of flowering tillers in the BCCIL population exhibits significant heritability in the narrow sense (S. Trinder, unpublished). Thus, the climate-driven genetic differentiation in P1 plant phenotypes that we have observed is consistent with an underlying evolutionary response.

The differences in germination timing that we have observed were measured under common environmental conditions on sexually produced offspring that had never been exposed to the environment at Buxton. Furthermore, their P1 parent plants had been grown in a common environment for three years prior to seed collection, during which time they had each produced many dozens of new tillers, minimizing the likelihood of carry-over effects on the phenotype (Schwaegerle, McIntyre, & Swingley, 2000). Some authors, however, have suggested that seed traits, and in particular, seed mass, are so strongly influenced by maternal effects that they can be viewed as a phenotype of the mother, not the offspring plant (Galloway, Etterson, & McGlothlin, 2009; Thiede, 1998). In our study, we found that F1 hybrid individuals had a germination timing phenotype intermediate to that of F1 individuals whose parents had both come from a single treatment at BCCIL. Mean phenotypes for the two reciprocal hybrid crosses were similar (Figure S3). This suggests that differences in germination timing were largely genetic in nature, since we would not expect this pattern if maternal effects contributed strongly to differences in germination phenotypes. Our data, however, do not allow us to identify specifically which type of gene effects are responsible, whether additive, dominance, or other (Lynch, 1991).

We argue that evolutionary change in *F. ovina* at BCCIL has involved multi-generational selection, and is not a consequence of the sorting of clones that predated the climate treatments at BCCIL. *F. ovina* has increased in abundance on drought-treated plots, but we have never detected the same plant genotype twice, in any of the climate treatments, as might be expected if surviving clones had expanded to drive increased abundance (Fridley et al., 2011; Ravenscroft et al., 2015). Therefore, recruitment by seed in *F. ovina* is likely to be a critical determinant of population size at BCCIL, while genetic change mediated by the expansion of established clones is likely to be less important.

Finally, neither gene flow between the plots at BCCIL nor genetic drift are able to explain the phenotypic changes we have documented. Analysis of amplified fragment length polymorphism data from BCCIL suggests that gene flow between plots is high ($F_{ST} = 0.006$; Ravenscroft et al., 2015). Such gene flow is expected to erode genetic and phenotypic differentiation between treatments (Ravenscroft et al., 2015). Any climatic selection driving evolutionary change at BCCIL must therefore be sufficiently strong to overcome this homogenizing force. Extensive gene flow between plots also rules out a role for genetic drift in differentiating plant phenotypes. Thus, we argue that the drought-induced phenotypic differentiation

we have observed arises from underlying genetic changes, and represent a true climate-driven evolutionary response.

4.2 | Evolutionary changes in reproductive success

Our results demonstrate that male reproductive success has decreased in response to long-term experimental drought manipulations at BCCIL by 37.1% relative to control plants. Plants from the drought-treated plots produced fewer flowering tillers than those from the control plots. Thus, one possible explanation for the observed reduction in male fitness is that the reduction in flowering tillers resulted in a lower total quantity of pollen, if pollen quantity is correlated with the number of flowering tillers (McKone, 1990). This would reduce the chances of pollen being transferred to the receptive stigma of another plant. However, other aspects of male function, such as pollen tube growth rate and pollen germination, may also have evolved in response to long-term drought, contributing to the observed reduction in male reproductive success (Hedhly, Hormaza, & Herrero, 2009; Schaeffer, Manson, & Irwin, 2013).

The number of flowering tillers and male reproductive success were positively associated, but there was considerable residual variation in male reproductive success (Figure 1c). In particular, four plants showed male reproductive success far greater than expected based on the number of flowering tillers that they produced. This finding emphasizes the need for direct measurements of male reproductive success, and suggests that flower number is unlikely to be a good proxy for this trait at the individual level (Snow & Lewis, 1993).

In this study, we did not have measures of female reproductive success through seed output because our design was balanced with respect to the number of seeds from each maternal parent. Hence, we cannot compare the relative contribution of male and female reproductive success to total fitness or assess whether the responses we observed have altered the contribution of male and female components to reproductive success. Such estimates are now needed, both experimentally, and in the field, to form a complete view of climatic effects on the plant mating system (Barrett & Harder, 2017).

4.3 | Assortative mating and reproductive isolation

Assortative mating can increase the speed of evolutionary responses and provide a mechanism for partial reproductive isolation within populations (Fox, 2003; Weis et al., 2005). We found no evidence that plants that originated from the same climate treatment at BCCIL were more likely to mate with each other. However, we did find evidence for assortative mating that favoured plants with coincident flowering times. Since, at least in some years, there is a genetic difference in flowering time between drought and control populations, this assortative mating may contribute to partial reproductive isolation. However, such reproductive isolation cannot be operating strongly or continuously, because molecular genetic differentiation is low (Ravenscroft et al., 2015), and we did not detect any assortative mating by treatment. Comparable reproductive isolation has been observed in long-term nutrient-addition treatments at the Park Grass Experiment, in Rothamsted. Here, Snaydon and Davies (1976) found that different nutrient treatment regimes had driven evolutionary differentiation in the flowering time of the short-lived perennial grass *Anthoxanthum odoratum*. Silvertown, Servaes, Biss, and Macleod (2005) later demonstrated that selection on flowering time had reinforced reproductive isolation by shifting flowering time so that it limited gene flow between the plots. Another example of assortative mating has been documented in *B. rapa*, where it was shown that genetic variation in flowering time led to assortative mating (Weis & Kossler, 2004). Franks and Weis, (2009) found that the evolution of flowering time in *B. rapa* in response to a 5-year natural drought altered reproductive isolation between populations through phenological assortative mating. Our results provide a novel demonstration of assortative mating that is likely to be driven periodically by the long-term experimental drought treatment at BCCIL. They also suggest the potential for evolutionary changes to be reinforced during episodes of drought-induced selection on flowering time.

4.4 | Evolutionary change in germination traits

Our results suggest that germination timing has evolved in *F. ovina*, in response to long-term drought manipulation at BCCIL. Seeds whose P1 parents had both originated in the control treatment (control ancestry) were on average faster to germinate than those with hybrid (control × drought; drought × control) or drought ancestry. Parental ancestral climate was not associated with seed mass, but seed mass was associated with germination latency; lighter seeds had, on average, a longer time to germination.

To understand selection on germination traits, it is necessary to understand the context of seed germination in the field. In our study system, the germination of *F. ovina* follows a well-defined seasonal pattern. Seeds typically fall from established plants from June through to September, and are observed to germinate soon after, through August to December (Thompson & Grime, 1979). Thus, Thompson and Grime (1979) concluded that germinating *F. ovina* seeds take advantage of bare ground left by disturbance in the grassland following summer droughts and animal grazing. The ability of *F. ovina* to germinate under a wide range of temperatures facilitates germination during the end of summer and autumn when temperatures may be variable (Thompson & Grime, 1979). These natural seasonal dynamics imply that a longer delay to seed germination under drought conditions could be adaptive. Most seed produced by *F. ovina* in the drought treatment plots will fall during the drought treatment itself (July–August). The first precipitation following the drought treatment is likely to initiate seed germination. Soil moisture usually recovers to pre-drought levels, and levels observed in the control plots very rapidly (Fridley et al., 2011). A longer lag to germination may be adaptive in guarding against any marginal germination cues that occur during the drought treatment, when it would be dis-advantageous for germination to occur, or to allow time for soil

moisture to be fully replenished at the end of the drought treatment. We do not know whether the lag to germination we have observed in the laboratory is representative of that under field conditions, or whether it is associated with reduced sensitivity to marginal germination cues. Further field data would be required to resolve this issue and reveal to what extent evolutionary differences in germination timing are ecologically adaptive at BCCIL (Donohue, 2005).

An alternative explanation for evolutionary changes in germination timing is that they are the result of correlated evolution with another trait. For example, in *Campanulastrum americanum*, artificial selection for earlier flowering time resulted in the correlated evolution of a delayed germination time (Burgess, Etterson, & Galloway, 2007), implying that these traits may share a part of their genetic basis. Studies in *Arabidopsis thaliana* have also revealed pleiotropy between flowering time and germination. Specifically, the FLOWERING LOCUS C (FLC) gene is strongly associated with both traits (Chiang, Barua, Kramer, Amasino, & Donohue, 2009; Debieu et al., 2013). In our study, we have evidence for both drought-induced advancement in flowering time and delay in germination timing. However, we do not know whether either flowering time or germination lag is under direct- or indirect selection via pleiotropy. In either case, our results support the occurrence of climate-driven evolutionary change.

5 | CONCLUSIONS

Together, our results suggest that reproductive and early-acting life-history traits in *F. ovina* have evolved rapidly in response to simulated climate change at BCCIL. Male reproductive success has been reduced in plants from the drought treatments, as a result of evolutionary changes in reproductive tiller number. Our finding of assortative mating by flowering time indicates a mechanism by which partial reproductive isolation could develop between plants from the drought and control treatments at BCCIL, periodically reinforcing the development of climate-driven evolution. Finally, we found that chronic drought treatment at BCCIL has driven an increased lag to germination in *F. ovina*. We do not yet know whether this response is directly adaptive or the result of correlated evolution between germination timing and another trait. Together, our results demonstrate rapid evolutionary change driven by a long-term drought treatment, in a long-lived perennial plant. Such evolution may provide plant populations with a means to resist the effects of climate change.

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AUTHORS' CONTRIBUTIONS

R.W. and S.T. conceived the ideas and designed methodology; A.P.A. maintained field-based climate treatments at BCCIL; R.W. and A.P.A. collected and maintained parental P1 *F. ovina* material from BCCIL; S.T. collected and analysed the data; S.T. and R.W. led the writing of the manuscript. All authors contributed critically to manuscript drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data has been deposited with the NERC Environmental Information Data Centre: https://doi.org/10.5285/65c14df6-b762-441c-8d8d-9112f7350f03 (Trinder, Askew, & Whitlock, 2020).

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REFERENCES

Anderson, J. T., Inouye, D. W., McKinney, A. M., Colautti, R. I., & Mitchell-Olds, T. (2012). Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society B*, *279*, 3834–3852. https://doi.org/10.1098/rspb.2012.1051

Antonovics, J., & Bradshaw, A. D. (1970). Evolution in closely adjacent plant populations VIII. Clinal patterns at a mine boundary. *Heredity*, *25*, 349–362. https://doi.org/10.1038/hdy.1970.36

Austen, E. J., & Weis, A. E. (2016). Estimating selection through male fitness: Three complementary methods illuminate the nature and causes of selection on flowering time. *Proceedings of the Royal Society B*, *283*, 20152635. https://doi.org/10.1098/rspb.2015.2635

Avolio, M. L., Beaulieu, J. M., & Smith, M. D. (2013). Genetic diversity of a dominant C(4) grass is altered with increased precipitation variability. *Oecologia*, *171*, 571–581. https://doi.org/10.1007/s00442-012-2427-4

Barrett, S. C. H., & Harder, L. D. (2017). The ecology of mating and its evolutionary consequences in seed plants. *Annual Reviews of Ecology, Evolution and Systematics*, *48*, 135–157. https://doi.org/10.1146/annurev-ecolsys-110316-023021

Baskin, C. C., & Baskin, J. M. (2014). *Seeds: Ecology, biogeography, and evolution of dormancy and germination* (2nd ed.). San Diego, CA: Academic Press.

Bertin, R. I. (1988). Paternity in plants. In J. Lovett-Doust & L. Lovett-Doust (Eds.), *Plant reproductive ecology: Patterns and strategies* (pp. 30–59). New York, NY: Oxford University Press.

Billington, H. L., & Pelham, J. (1991). Genetic variation in the date of budburst in Scottish birch populations: Implications for climate change. *Functional Ecology*, *5*, 403–409. https://doi.org/10.2307/2389812

Bilton, M. C., Whitlock, R., Grime, J. P., Marion, G., & Pakeman, R. J. (2010). Intraspecific trait variation in grassland plant species reveals fine-scale strategy trade-offs and size differentiation that underpins performance in ecological communities. *Botany-Botanique*, *88*, 939–952. https://doi.org/10.1139/B10-065

Burgess, K. S., Etterson, J. R., & Galloway, L. F. (2007). Artificial selection shifts flowering phenology and other correlated traits in an autotetraploid herb. *Heredity*, *99*, 641–648. https://doi.org/10.1038/sj.hdy.6801043

Chiang, G. C. K., Barua, D., Kramer, E. M., Amasino, R. M., & Donohue, K. (2009). Major flowering time gene, FLOWERING LOCUS C, regulates seed germination in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences*, *106*, 11661–11666. https://doi.org/10.1073/pnas.0901367106

Clark, L. V. (2014). polysat version 1.3 Tutorial Manual. Retrieved from http://openwetware.org/wiki/Polysat

Clark, L., & Jasieniuk, M. (2011). POLYSAT: An R package for polyploid microsatellite analysis. *Molecular Ecology Resources*, *11*, 562–566. https://doi.org/10.1111/j.1755-0998.2011.02985.x

Conner, J. K., Rush, S., Kercher, S., & Jennetten, P. (1996). Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). II. Selection through lifetime male and total fitness. *Evolution*, *50*, 1137–1146. https://doi.org/10.1111/j.1558-5646.1996.tb02354.x

Cook, B. I., Wolkovich, E. M., & Parmesan, C. (2012). Divergent responses to spring and winter warming drive community level flowering trends. *Proceedings of the National Academy of Sciences*, *109*, 9000–9005. https://doi.org/10.1073/pnas.1118364109

Debieu, M., Tang, C., Stich, B., Sikosek, T., Effgen, S., Josephs, E., … de Meaux, J. (2013). Co-variation between seed dormancy, growth rate and flowering time changes with latitude in *Arabidopsis thaliana*. *PLoS ONE*, *8*, e61075. https://doi.org/10.1371/journal.pone.0061075

Devlin, B., & Ellstrand, N. C. (1990). Male and female fertility variation in wild radish, a hermaphrodite. *The American Naturalist*, *136*, 87–107. https://doi.org/10.1086/285083

Donohue, K. (2005). Seeds and seasons: interpreting germination timing in the field. *Seed Science Research*, *15*, 175–187. https://doi.org/10.1079/ssr2005208

Donohue, K., de Casas, R. R., Burghardt, L., Kovach, K., & Willis, C. G. (2010). Germination, postgermination adaptation, and species ecological ranges. *Annual Review of Ecology and Systematics*, *41*, 293–319. https://doi.org/10.1146/annurev-ecolsys-102209-144715

Eckert, C. G., Kalisz, S., Geber, M. A., Sargent, R., Elle, E., Cheptou, P.-O., … Winn, A. A. (2010). Plant mating systems in a changing world. *Trends in Ecology & Evolution*, *25*, 35–43. https://doi.org/10.1016/j.tree.2009.06.013

Ennos, R. A., & Dodson, R. K. (1987). Pollen success, functional gender and assortative mating in an experimental plant population. *Heredity*, *58*, 119–126. https://doi.org/10.1038/hdy.1987.16

Etterson, J. R., & Mazer, S. J. (2016). How climate change affects plants' sex lives. *Science*, *353*, 32–33. https://doi.org/10.1126/science.aag1624

Fenner, M., & Thompson, K. (2005). *The ecology of seeds* (1st ed.). Cambridge: Cambridge University Press.

Fitter, A. H., & Fitter, R. S. R. (2002). Rapid changes in flowering time in British plants. *Science*, *296*, 1689–1691. https://doi.org/10.1126/science.1071617

Fox, G. A. (2003). Assortative mating and plant phenology: Evolutionary and practical consequences. *Evolutionary Ecology Research*, *5*, 1–18.

Franks, S. J., Sim, S., & Weis, A. E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences*, *104*, 1278–1282. https://doi.org/10.1073/pnas.0608379104

Franks, S. J., Weber, J. J., & Aitken, S. N. (2014). Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications*, *7*, 123–139. https://doi.org/10.1111/eva.12112

Franks, S. J., & Weis, A. E. (2009). Climate change alters reproductive isolation and potential gene flow in an annual plant. *Evolutionary Applications*, *2*, 481–488. https://doi.org/10.1111/j.1752-4571.2009.00073.x

Fridley, J. D., Grime, J. P., Askew, A. P., Moser, B., & Stevens, C. J. (2011). Soil heterogeneity buffers community response to climate change in species-rich grassland. *Global Change Biology*, *17*, 2002–2011. https://doi.org/10.1111/j.1365-2486.2010.02347.x

Fridley, J. D., Lynn, J. S., Grime, J. P., & Askew, A. P. (2016). Longer growing season shifts grassland vegetation towards more-productive species. *Nature Climate Change*, *6*, 865–868. https://doi.org/10.1038/nclimate3032

Galloway, L. F., Etterson, J. R., & McGlothlin, J. W. (2009). Contribution of direct and maternal genetic effects to life-history evolution. *New Phytologist*, *183*, 826–838. https://doi.org/10.1111/j.1469-8137.2009.02939.x

Ghatnekar, L. (1999). A polymorphic duplicated locus for cytosolic PGI segregating in sheep's fescue (*Festuca ovina* L.). *Heredity*, *83*, 451–459. https://doi.org/10.1038/sj.hdy.6885750

Gonzalo-Turpin, H., & Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology*, *97*, 742–751. https://doi.org/10.1111/j.1365-2745.2009.01509.x

Grime, J. P., Brown, V. K., Thompson, K., Masters, G. J., Hillier, S. H., Clarke, I. P., … Kielty, J. P. (2000). The response of two contrasting limestone grasslands to simulated climate change. *Science*, *289*, 762–765. https://doi.org/10.1126/science.289.5480.762

Grime, J. P., Fridley, J. D., Askew, A. P., Thompson, K., Hodgson, J. G., & Bennett, C. R. (2008). Long-term resistance to simulated climate change in an infertile grassland. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 10028–10032. https://doi.org/10.1073/pnas.0711567105

Guilioni, L., Wéry, J., & Lecoeur, J. (2003). High temperature and water deficit may reduce seed number in field pea purely by decreasing plant growth rate. *Functional Plant Biology*, *30*, 1151–1164. https://doi.org/10.1071/FP03105

Hadfield, J. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCGLMM R package. *Journal of Statistical Software*, *33*, 1–22. https://doi.org/10.18637/jss.v033.i02

Hadfield, J. D., Richardson, D. S., & Burke, T. (2006). Towards unbiased parentage assignment: Combining genetic, behavioural and spatial data in a Bayesian framework. *Molecular Ecology*, *15*, 3684. https://doi.org/10.1111/j.1365-294X.2006.03050.x

Hedhly, A., Hormaza, J. I., & Herrero, M. (2009). Global warming and sexual plant reproduction. *Trends in Plant Science*, *14*, 30–36. https://doi.org/10.1016/j.tplants.2008.11.001

Hoffmann, A. A., & Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*, 479–485. https://doi.org/10.1038/nature09670

Jóhannsson, M. H., & Stephenson, A. G. (1998). Effects of temperature during microsporogenesis on pollen performance in *Cucurbita pepo* L. (Cucurbitaceae). *International Journal of Plant Sciences*, *159*, 616–626. https://doi.org/10.1086/297580

Jump, A. S., Peñuelas, J., Rico, L., Ramallo, E., Estiarte, M., Martinez-Izquierdo, J. A., & Lloret, F. (2008). Simulated climate change provokes rapid genetic change in the Mediterranean shrub *Fumana thymifolia*. *Global Change Biology*, *14*, 637–643. https://doi.org/10.1111/j.1365-2486.2007.01521.x

Lau, T.-C., & Stephenson, A. G. (1993). Effects of soil nitrogen on pollen production, pollen grain size, and pollen performance in *Cucurbita pepo* (Cucurbitaceae). *American Journal of Botany*, *80*, 763–768. https://doi.org/10.1002/j.1537-2197.1993.tb15292.x

Lau, T.-C., & Stephenson, A. G. (1994). Effects of soil phosphorus on pollen production, pollen size, pollen phosphorus content, and the ability to sire seeds in *Cucurbita pepo* (Cucurbitaceae). *Sexual Plant Reproduction*, *7*, 215–220. https://doi.org/10.1007/BF00232740

Lynch, M. (1991). The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution*, *45*, 622–629. https://doi.org/10.1111/j.1558-5646.1991.tb04333.x

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits*. Sunderland, MA, USA: Sinauer Associates Inc.

Marshall, D. L., Tyler, A. P., Abrahamson, N. J., Avritt, J. J., Barnes, M. G., Larkin, L. L., … Maliakal-Witt, S. (2010). Pollen performance of *Raphanus sativus* (Brassicaceae) declines in response to elevated [CO_2]. *Sexual Plant Reproduction*, *23*, 325–336. https://doi.org/10.1007/s00497-010-0146-8

McKone, M. J. (1990). Characteristics of pollen production in a population of New Zealand snow-tussock grass (*Chionochloa pallens* Zotov). *New Phytologist*, *116*, 555–562. https://doi.org/10.1111/j.1469-8137.1990.tb00539.x

McNeilly, T., & Antonovics, J. (1968). Evolution in closely adjacent plant populations IV. *Barriers to Gene Flow. Heredity*, *23*, 205–218. https://doi.org/10.1038/hdy.1968.29

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., … Zust, A. (2006). European phenological response to climate change matches the warming pattern. *Global Change Biology*, *12*, 1969–1976. https://doi.org/10.1111/j.1365-2486.2006.01193.x

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, *7*, 1–14. https://doi.org/10.1111/eva.12137

Munguía-Rosas, M. A., Ollerton, J., Parra-Tabla, V., & De-Nova, J. A. (2011). Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecology Letters*, *14*, 511–521. https://doi.org/10.1111/j.1461-0248.2011.01601.x

Nevo, E., Fu, Y.-B., Pavlíček, T., Khalifa, S., Tavasi, M., & Beiles, A. (2012). Evolution of wild cereals during 28 years of global warming in Israel. *Proceedings of the National Academy of Sciences*, *109*, 3412–3415. https://doi.org/10.1073/pnas.1121411109

Parmesan, C., & Hanley, M. E. (2015). Plants and climate change: Complexities and surprises. *Annals of Botany*, *116*, 849–864. https://doi.org/10.1093/aob/mcv169

Pasonen, H.-L., Pulkkinen, P., & Kärkkäinen, K. (2002). Genotype-environment interactions in pollen competitive ability in an anemophilous tree, *Betula pendula* Roth. *Theoretical and Applied Genetics*, *105*, 465–473. https://doi.org/10.1007/s00122-002-0944-y

Poulton, J. L., Koide, R. T., & Stephenson, A. G. (2001). Effects of mycorrhizal infection, soil phosphorus availability and fruit production on the male function in two cultivars of *Lycopersicon esculentum*. *Plant, Cell & Environment*, *24*, 841–849. https://doi.org/10.1046/j.0016-8025.2001.00735.x

Prasad, P. V. V., Staggenborg, S. A., & Ristic, Z. (2008). Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In L. Ahuja, V. R. Reddy, S. A. Saseendran, & Q. Yu (Eds.), *Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes*. Madison, WI: American Society of Agronomy, Inc; Crop Science Society of America, Inc; Soil Science Society of America Inc.

Primack, R. B., & Kang, H. (1989). Measuring fitness and natural selection in wild plant populations. *Annual Review of Ecology and Systematics*, *20*, 367–396. https://doi.org/10.1146/annurev.ecolsys.20.1.367

R Development Core Team. (2008). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ravenscroft, C. H., Fridley, J. D., & Grime, J. P. (2014). Intraspecific functional differentiation suggests local adaptation to long-term climate change in a calcareous grassland. *Journal of Ecology*, *102*, 65–73. https://doi.org/10.1111/1365-2745.12168

Ravenscroft, C., Whitlock, R., & Fridley, J. (2015). Rapid genetic divergence in response to 15 years of simulated climate change. *Global Change Biology*, *21*, 4165–4176. https://doi.org/10.1111/gcb.12966

Schaeffer, R. N., Manson, J. S., & Irwin, R. E. (2013). Effects of abiotic factors and species interactions on estimates of male plant function: A meta-analysis. *Ecology Letters*, *16*, 399–408. https://doi.org/10.1111/ele.12044

Schwaegerle, K. E., McIntyre, H., & Swingley, C. (2000). Quantitative genetics and the persistence of environmental effects in clonally propagated organisms. *Evolution*, *54*, 452–461. https://doi.org/10.1111/j.0014-3820.2000.tb00048.x

Silvertown, J., Servaes, C., Biss, P., & Macleod, D. (2005). Reinforcement of reproductive isolation between adjacent populations in the Park Grass Experiment. *Heredity*, *95*, 198–205. https://doi.org/10.1038/sj.hdy.6800710

Snaydon, R. W., & Davies, M. S. (1972). Rapid population differentiation in a mosaic environment. II Morphological variation in *Anthoxanthum odoratum*. *Evolution*, *26*, 390–405. https://doi.org/10.2307/2407014

Snaydon, R. W., & Davies, M. S. (1976). Rapid population differentiation in a mosaic environment IV. Populations of *Anthoxanthum odoratum* at sharp boundaries. *Heredity*, *37*, 9–25. https://doi.org/10.1038/hdy.1976.62

Snow, A. A., & Lewis, P. O. (1993). Reproductive traits and male fertility in plants: Empirical approaches. *Annual Review of Ecology and Systematics*, *24*, 331–351. https://doi.org/10.1146/annurev.es.24.110193.001555

Stace, C. (2010). *New flora of the British Isles*. Cambridge, UK: Cambridge University Press.

Sultan, S. E., Horgan-Kobelski, T., Nichols, L. M., Riggs, C. E., & Waples, R. K. (2013). A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. *Evolutionary Applications*, *6*, 266–278. https://doi.org/10.1111/j.1752-4571.2012.00287.x

Therneau, T. M., & Grambsch, P. M. (2000). *Modeling survival data: Extending the Cox model*. New York, NY: Springer.

Thiede, D. A. (1998). Maternal inheritance and its effect on adaptive evolution: A quantitative genetic analysis of maternal effects in a natural plant population. *Evolution*, *52*, 998–1015. https://doi.org/10.1111/j.1558-5646.1998.tb01829.x

Thompson, K., & Grime, J. P. (1979). Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology*, *67*, 893–921. https://doi.org/10.2307/2259220

Trinder, S., Askew, A. P., & Whitlock, R. (2020). Data from: Reproductive and early-acting life-history traits in the perennial grass Festuca ovina exposed to drought conditions. *NERC Environmental Information Data Centre*, https://doi.org/10.5285/65c14df6-b762-441c-8d8d-9112f7350f03

Waits, L. P., Luikart, G., & Taberlet, P. (2001). Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Molecular Ecology*, *10*, 249–256. https://doi.org/10.1046/j.1365-294X.2001.01185.x

Walck, J. L., Hidayati, S. N., Dixon, K. W., Thompson, K., & Poschlod, P. (2011). Climate change and plant regeneration from seed. *Global Change Biology*, *17*, 2145–2161. https://doi.org/10.1111/j.1365-2486.2010.02368.x

Wang, X., Taub, D. R., & Jablonski, L. M. (2015). Reproductive allocation in plants as affected by elevated carbon dioxide and other environmental changes: A synthesis using meta-analysis and graphical vector analysis. *Oecologia*, *177*, 1075–1087. https://doi.org/10.1007/s00442-014-3191-4

Weilbull, P., Ghatnekar, L., & Bengtsson, B. O. (1991). Genetic variation in commercial varieties and natural populations of sheep's fescue, *Festuca ovina s.l*. *Plant Breeding*, *107*, 203–209. https://doi.org/10.1111/j.1439-0523.1991.tb01207.x

Weis, A. E., & Kossler, T. M. (2004). Genetic variation in flowering time induces phenological assortative mating: Quantitative genetic methods applied to *Brassica rapa*. *American Journal of Botany*, *91*, 825–836. https://doi.org/10.3732/ajb.91.6.825

Weis, A. E., Winterer, J., Vacher, C., Kossler, T. M., Young, C. A., & LeBuhn, G. L. (2005). Phenological assortative mating in flowering plants: The nature and consequences of its frequency dependence. *Evolutionary Ecology Research*, *7*, 161–181.

Whitlock, R., Hipperson, M., Mannarelli, M., & Burke, T. (2008). A high-throughput protocol for extracting high-purity genomic DNA from plants and animals. *Molecular Ecology Resources*, *8*, 736–741. https://doi.org/10.1111/j.1755-0998.2007.02074.x

Wright, S. (1921). Systems of mating. III. Assortative mating based on somatic resemblence. *Genetics*, *6*, 144–161.

Young, H. J., & Stanton, M. L. (1990). Influence of environment quality on pollen competitive ability in wild radish. *Science*, *248*, 1631–1633. https://doi.org/10.1126/science.248.4963.1631

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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