



A natural heating experiment: Phenotypic and genotypic responses of plant phenology to geothermal soil warming

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Keywords:	plant phenology, climatic variation, geothermal ecosystems, Iceland, microclimate, phenotypic plasticity, local adaptation, phenotypic selection
Abstract:	<p>Under global warming, the survival of many populations of sedentary organisms in seasonal environments will largely depend on their ability to cope with warming in situ by means of phenotypic plasticity or adaptive evolution. This is particularly true in high-latitude environments, where current growing seasons are short, and expected temperature increases large. In such short-growing season environments, the timing of growth and reproduction is critical to survival. Here, we use the unique setting provided by a natural geothermal soil warming gradient (Hengill geothermal area, Iceland) to study the response of <i>Cerastium fontanum</i> flowering phenology to temperature. We hypothesized that trait expression and phenotypic selection on flowering phenology are related to soil temperature, and tested the hypothesis that temperature-driven differences in selection on phenology have resulted in genetic differentiation using a common garden experiment. In the field, phenology was related to soil temperature, with plants in warmer microsites flowering earlier than plants at colder microsites. In the common garden, plants responded to spring warming in a counter-gradient fashion; plants originating from warmer microsites flowered relatively later than those originating from colder microsites. A likely explanation for this pattern is that plants from colder microsites have been selected to compensate for the shorter growing season by starting development at lower temperatures. However, in our study we did not find evidence of variation in phenotypic selection on phenology in relation to temperature, but selection consistently favoured early flowering. Our results show that soil temperature influences trait expression and suggest the existence of genetically-based variation in flowering phenology leading to counter-</p>

	gradient local adaptation along a gradient of soil temperatures. An important implication of our results is that observed phenotypic responses of phenology to global warming might often be a combination of short-term plastic responses and long-term evolutionary responses, acting in opposite directions.

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13 ABSTRACT

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15 environments will largely depend on their ability to cope with warming *in situ* by means of
16 phenotypic plasticity or adaptive evolution. This is particularly true in high-latitude
17 environments, where current growing seasons are short, and expected temperature increases
18 large. In such short-growing season environments, the timing of growth and reproduction is
19 critical to survival. Here, we use the unique setting provided by a natural geothermal soil
20 warming gradient (Hengill geothermal area, Iceland) to study the response of *Cerastium*
21 *fontanum* flowering phenology to temperature. We hypothesized that trait expression and
22 phenotypic selection on flowering phenology are related to soil temperature, and tested the
23 hypothesis that temperature-driven differences in selection on phenology have resulted in
24 genetic differentiation using a common garden experiment. In the field, phenology was
25 related to soil temperature, with plants in warmer microsites flowering earlier than plants at
26 colder microsites. In the common garden, plants responded to spring warming in a counter-
27 gradient fashion; plants originating from warmer microsites flowered relatively later than
28 those originating from colder microsites. A likely explanation for this pattern is that plants
29 from colder microsites have been selected to compensate for the shorter growing season by
30 starting development at lower temperatures. However, in our study we did not find evidence
31 of variation in phenotypic selection on phenology in relation to temperature, but selection
32 consistently favoured early flowering. Our results show that soil temperature influences trait
33 expression and suggest the existence of genetically-based variation in flowering phenology
34 leading to counter-gradient local adaptation along a gradient of soil temperatures. An
35 important implication of our results is that observed phenotypic responses of phenology to
36 global warming might often be a combination of short-term plastic responses and long-term
37 evolutionary responses, acting in opposite directions.

38 INTRODUCTION

39 Climate change is causing significant increases in temperature across the world, with
40 warming being most pronounced towards Arctic regions (IPCC, 2014), and with important
41 effects on biodiversity (Bellard, Bertelsmeier, Leadley, Thuiller, & Courchamp, 2012). .
42 Species responses to global warming (Parmesan, 2006; Parmesan & Yohe, 2003) might
43 involve distributional changes to track appropriate conditions in space (range shifts), or
44 changes in the timing of life cycle events (phenological shifts) to keep up with seasonally
45 changing abiotic factors. Most observations of responses to climate change have concerned
46 alterations in phenology (E. E. Cleland, Chuine, Menzel, Mooney, & Schwartz, 2007; Ibáñez
47 et al., 2010; Parmesan, 2006), and such responses should be critical for survival at high
48 latitudes, where the possibilities for dispersal to colder areas are restricted. Individuals can
49 change their phenology in response to warming through phenotypic plasticity (i.e. the ability
50 of a given genotype to express different phenotypes in different environments, Agrawal, 2001)
51 and populations can respond evolutionary through changes in their genetic structure
52 (Anderson, Inouye, McKinney, Colautti, & Mitchell-Olds, 2012; Franks, Weber, & Aitken,
53 2014). Observed phenotypic responses to warming have often been argued to be mainly due
54 to phenotypic plasticity (Hoffmann & Sgrò, 2011; Leblans et al., 2017; Merilä & Hendry,
55 2014), and only to a smaller extent due to evolutionary changes (Franks, Sim, & Weis, 2007;
56 Lavergne, Mouquet, Thuiller, & Ronce, 2010).

57 The seasonal timing of life cycle events determines how an organism interacts with
58 the surrounding environment, and variation in phenology can have strong effects on survival
59 and reproduction. Higher spring temperatures caused by current climate change have been
60 shown to cause shifts in timing and length of the growing season, with an earlier start being
61 responsible for most of these shifts (Linderholm, 2006). Higher temperatures and the
62 subsequent advancement of the growing season might condition the timing of life cycle events

of an organism in a given year, leading to phenotypic plasticity in phenology as a consequence of direct effects of climate on physiological and developmental rates. However, variation in climate can also influence natural selection on timing, and differential selection might lead to genetic differentiation of populations (Anderson et al., 2012; Franks et al., 2007; Visser, 2008). Local adaptation through genetic responses along climatic gradients can be either co-gradient, when genetic and environmental influences on a phenotype act in the same direction, counter-gradient when they act in opposite directions, or show no relation with the climatic gradient (Conover & Schultz, 1995). Counter-gradient variation would be expected if growing season length is the only factor influencing selection on phenology. In this case, cold environments with short growing seasons (e.g. high latitudes or altitudes) would select for a higher sensitivity of developmental rates to spring warming, leading to the evolution of faster developing genotypes in cold environments than in warm environments. Individuals from cold environments would thus be able to start development at lower temperatures than individuals from warm environments when grown under similar conditions during spring.

The effects of climate warming on plant phenology have usually been examined empirically, by examining patterns of local adaptation along latitudinal (Toftegaard et al., 2016) or altitudinal gradients (Frei, Ghazoul, Matter, Heggli, & Pluess, 2014), or by performing experiments using open-top chambers or other warming devices (Arft et al., 1999; Wolkovich et al., 2012). Despite the convenience of latitudinal and altitudinal gradients as natural set-ups to study species' responses to climate warming (De Frenne et al., 2013; Körner, 2007), they suffer from the limitation that other environmental factors often covary with temperature along these gradients. Experimental manipulation of temperatures allows us to dissect temperature effects from confounding environmental factors that covary with temperature in natural conditions. However, experiments are usually performed at smaller spatial scales, and are often associated with unintended disturbances in abiotic or biotic

conditions (e.g. light availability, wind speed, soil moisture, biotic interactions) caused by environmental manipulations, that can result in the underprediction of phenological responses to warming (Wolkovich et al., 2012). Moreover, the relatively short duration of most experiments does not allow the examination of long-term evolutionary responses. Geothermally heated ecosystems (Leblans et al., 2017; O’Gorman et al., 2014) provide unique “natural laboratories” for studying long-term responses to temperature increase, avoiding many of the limitations of both larger-scale temperature gradients and experimental designs. As some of these systems have been warmed for a long time (e.g. more than 50 years of continuous warming on Icelandic sub-arctic grasslands, Leblans et al., 2017; O’Gorman et al., 2014), they allow the evaluation of the long-term effects of warming on plant phenology. Moreover, temperature differences in these systems are large (e.g. 0-50 °C, O’Gorman et al., 2014), and occur over small spatial scales, reducing variation in other environmental factors (Richardson, Urban, Bolnick, & Skelly, 2014; Leblans et al., 2017; Robinson, McLaughlin, Marteinsdóttir, & O’Gorman, 2018). Combinations of observational studies on natural geothermal soil warming gradients with common garden experiments should thus constitute a very powerful approach to assess plastic and genetic responses of plant phenology to warming.

In this study, we investigated the relationship between small-scale variation in geothermal soil warming and phenotypic and genotypic differences in flowering phenology, using the perennial herb *Cerastium fontanum* in sub-arctic Icelandic grasslands where large differences in growing season length along natural geothermal soil warming gradients have been documented (Leblans et al., 2017; Perron, 2017). Because we expected differences in other environmental factors to be small, we predicted that growing season length should strongly influence selection on plant flowering phenology, leading to counter-gradient variation (Conover & Schultz, 1995) in genetic responses to warming. More specifically, we

113 hypothesized that: 1) colder microsites are associated with a later flowering phenology in the
114 field, 2) natural selection for early flowering is stronger at colder microsites with shorter
115 growing seasons, and as a consequence of such differences in selection, 3) there are
116 genetically based differences in phenology that are related to soil temperature at the microsite
117 of origin, and these differences are in a counter-gradient fashion, with plants originating from
118 colder microsites flowering earlier.

119 MATERIALS AND METHODS

120 **Study system**

121 The study was carried out in the Hengill geothermal area, 40 km east of Reykjavik, Iceland
122 (64° 3' 11"N, 21° 18' 16"W; 360 m a.s.l.). This area is located at the base of the Hengill
123 volcanic system, near to the Hrómundartindur and Hveragerði volcanic systems, where the
124 Reykjanes Volcanic Zone, the West Volcanic Zone and the South Iceland Seismic Zone
125 converge (Saemundsson, 1992; Zakharova & Spichak, 2012). The underlying bedrock of the
126 study site contains geothermal channels originating from high volcanic activity common at
127 tectonic boundaries (Zakharova & Spichak, 2012). These channels warm the water and soil
128 through radiative heating (Gudmundsdottir et al., 2011; Saemundsson, 1995). The soil
129 temperatures range from average ambient to over 20°C above ambient in some areas with
130 little to no warming on the air temperature (O’Gorman et al., 2014). The study area covers
131 approximately 0.5 km² where the main vegetation type consists of unmanaged, but grazed,
132 subarctic grassland. Grassland ecosystems cover ca. 40% of the global terrestrial surface (of
133 which 25% is at northern high latitudes, Chapin III, Matson, & Vitousek, 2011), and
134 temperature has been suggested to be an important driver of grassland phenology (E. E.
135 Cleland, Chiariello, Loarie, Mooney, & Field, 2006; Frei et al., 2014). Apart from soil
136 temperature, there are no significant changes in other abiotic factors (e.g. soil chemistry,
137 elevation) in our study area (Robinson et al., 2018). This system has been heated for over 50

138 years (probably much longer, (Saemundsson, 1967), offering a unique natural laboratory for
139 studying the long-term effects of warming.

140 For this study, we focused on the effects of warming on flowering phenology of
141 *Cerastium fontanum*, a short-lived clonal perennial herb that flowers in June and July in the
142 study area. It can be cross or self-pollinated and it shows unspecialized seed dispersal
143 (<http://ecoflora.org.uk>, Kristinsson, 1998). We chose this study species because it occurs over
144 a relatively wide range of temperatures, and because it can be easily grown from seeds.

145 **Data collection**

146 We collected data on soil temperature and flowering phenology in 2015 (100 plants over an
147 area of 0.25 km²) and 2017 (420 plants over an area of 0.5 m²). In each of these two years,
148 plant individuals were marked before the start of flowering and followed throughout the entire
149 season. The marked plants were different in 2015 and 2017. Soil temperature was measured
150 once in the immediate vicinity (< 2 cm) of each of the plants with a soil thermometer.
151 Temperature was measured at a depth of 10 cm, i.e. at the level of the plant roots, which is
152 little affected by air temperature. Therefore, these temperature measurements reflect long-
153 term differences in soil temperature, and are not influenced by weather during the day of
154 measurement. Measures of plant phenology differed among the two study years. In 2015,
155 flowering phenology was estimated based on the reproductive development at the day of
156 recording (between June 30 and July 3), and coded as a binomial variable, being 1 in plants
157 that had no open flowers, and 0 in plants that had at least 1 flower open on that day. In 2017,
158 plant reproductive development was followed on a regular basis from the beginning of June,
159 and first flowering date (FFD, the date when the first open flower was observed) was used as
160 an estimate of flowering phenology. In both years, higher values of the phenology variable
161 represent a later flowering phenology. In 2017, the numbers of flowers and fruits per plant
162 were also counted, and a sample of 1-8 fruits was collected. The number of seeds in these

fruits was counted afterwards in the lab, and the number of seeds per plant was calculated by multiplying the number of fruits per plant by the mean number of seeds per fruit.

To extract seeds for sowing, we collected up to 3 fruits from each marked plant in 2015. The seeds were sown in the greenhouse in January 2016, and plants were transferred to a common garden at Stockholm University (mean annual air temperature = 8°C for 2017) in June 2016. None of the plants flowered in 2016. In 2017, reproductive development of these plants in the common garden was followed on a regular basis from the beginning of June, and FFD (converted to Julian date) was used as an estimate of flowering phenology. A total of 540 plants corresponding to 98 maternal families survived and flowered in the common garden in 2017, and the number of flowering offspring per mother plant (i.e. plant flowering in the field from which seeds were collected) ranged from 1 to 10 plants (mean \pm SD = 5.5 \pm 2.7).

Statistical analyses

To assess if local soil temperature is associated with an earlier phenology in the field, we fitted generalized linear models of flowering phenology against soil temperature in both study years. We used a binomial model for 2015 (as phenology was measured as a binomial response variable, non-flowering vs. flowering at the time of recording) and a linear model for 2017 (as FFD showed an approximately normal distribution).

To test if phenotypic selection on flowering phenology depended on soil temperature, we performed a linear selection gradient analysis (Lande & Arnold, 1983) with the data from 2017. Fitness, in terms of the number of seeds per plant, was used as the response variable, and phenology, flower number, soil temperature and the interaction phenology \times soil temperature were included as fixed predictors. Flower number was included as a condition trait, i.e. a trait which is likely to be correlated with plant resource state, in order to get better estimates of selection on phenology, and to reduce the bias caused by environmental covariance (Rausher, 1992). Fitness was related by dividing each value by the mean, and

188 phenology and flower number were standardized by subtracting their mean and dividing by
189 their standard deviation. We also run the same model without the interaction term in order to
190 assess the overall direction of selection on phenology.

191 To assess if phenology in a common environment differs among maternal families, and
192 is related to soil temperature at the microsite of origin of the mother plant, we first tested if
193 flowering phenology in the common garden in 2017 differed among plants with different
194 mothers. For this, we fitted a linear mixed model with phenology in the common garden (FFD
195 converted to Julian date) as the response variable and mother as random effect. As we found
196 significant differences in phenology among plants with different mothers (see results), we
197 then evaluated if these differences were related to soil temperature at the origin of the mother
198 plant by fitting another linear mixed model with phenology in the common garden as the
199 response, soil temperature at the origin as a fixed effect, and mother as random effect. In each
200 case, we tested the significance of the random effect using a likelihood ratio test, comparing a
201 model with the random effect to the same model without the random effect.

202 Statistical analyses were carried out in R ver. 3.5.0 (R Core Team, 2018).

203 RESULTS

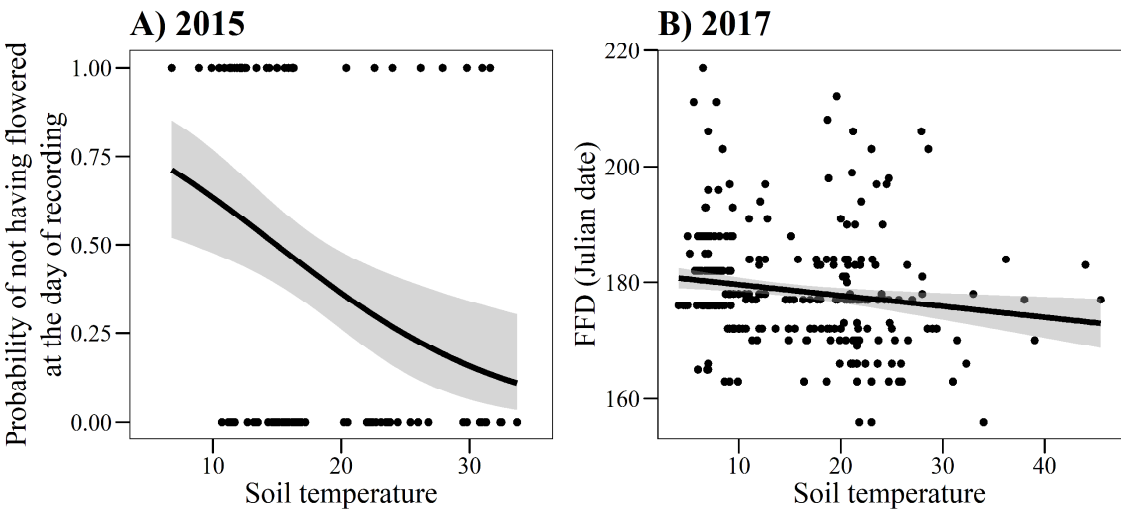
204 Soil temperature ranged from 6.8 to 33.7 °C in 2015, with a mean value of 18.2 °C, and from
205 4.1 to 45.5 °C in 2017, with a mean value of 14.4 °C. Soil temperature showed a large
206 variation within short distances; soil temperatures of plants located 1 m apart sometimes
207 differed by 10 °C, and plants located 10 m apart differed with up to 25 °C.

208 Higher soil temperature was associated with an earlier phenology in both study years
209 (Table 1). In 2015, the probability of not having flowered at the day of recording decreased
210 with soil temperature (Fig. 1A), and in 2017, FFD was earlier with higher soil temperatures
211 (Fig. 1B).

Table 1: Results of generalized linear models assessing the effects of soil temperature on *Cerastium fontanum* flowering phenology, measured as the probability of not having flowered at the day of recording in 2015 (binomial model) and as the first flowering date (converted to Julian date) in 2017 (linear model).

Year	N	Estimate	SE	z/t	P
2015	96	-0.111	0.036	-3.11	0.002
2017	300	-0.189	0.068	-2.80	0.006

Figure 1: Effect of soil temperature on flowering phenology of *Cerastium fontanum*, measured as (A) the probability of not having flowered at the day of recording in 2015 and (B) the first flowering date (FFD) converted to Julian date in 2017. In both cases, higher values representing a later flowering phenology. Lines show a binomial model fit in A, and a linear model fit in B.

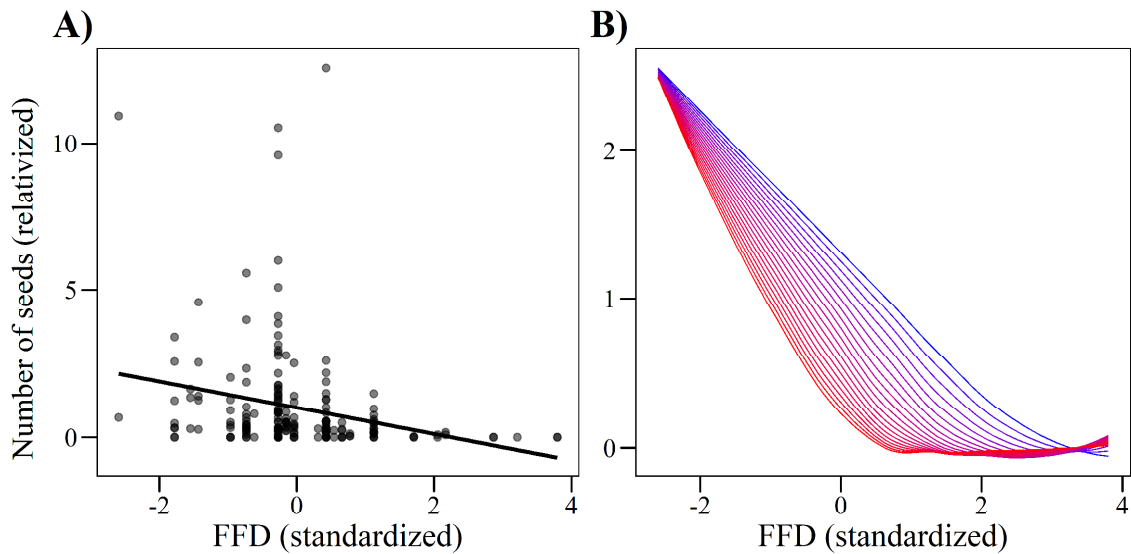


The relationship between plant phenology and fitness did not depend on soil temperature (the effect of the interaction phenology \times soil temperature was not significant, Table 2A, Fig. 2B). When the interaction was removed from the model, fitness increased with an earlier flowering (Table 2B, Fig. 2A).

Table 2: A) Results of a selection gradient analysis assessing the effects of flowering phenology (first flowering date) and flower number of *Cerastium fontanum*, as well as soil temperature and the interaction phenology \times soil temperature on plant fitness in 2017 (N = 193 plants where both traits and fitness data was available). B) Results of a similar selection gradient analysis without the interaction term. Fitness was estimated by the number of seeds per plant and relativized before analyses. Phenology and number of flowers were standardized before analyses.

	Estimate	SE	t	P
A) Model with interaction phenology \times soil temperature				
Phenology	-0.423	0.275	-1.54	0.126
Number of flowers	-0.160	0.135	-1.18	0.239
Soil temperature	-0.041	0.020	-2.11	0.036
Phenology : Soil temperature	-0.014	0.018	-0.76	0.446
B) Model without interaction phenology \times soil temperature				
Phenology	-0.603	0.143	-4.23	<0.001
Number of flowers	-0.171	0.134	-1.27	0.205
Soil temperature	-0.037	0.019	-1.98	0.049

234 Figure 2: Effect of standardized first flowering date (FFD) on relative fitness of *Cerastium*
235 *fontanum*, measured as the relative number of seeds per plant. (A) Linear model fit for the
236 overall relationship. (B) Linear model fits for different soil temperatures, from cold (blue) to
237 warm (red) microsites.

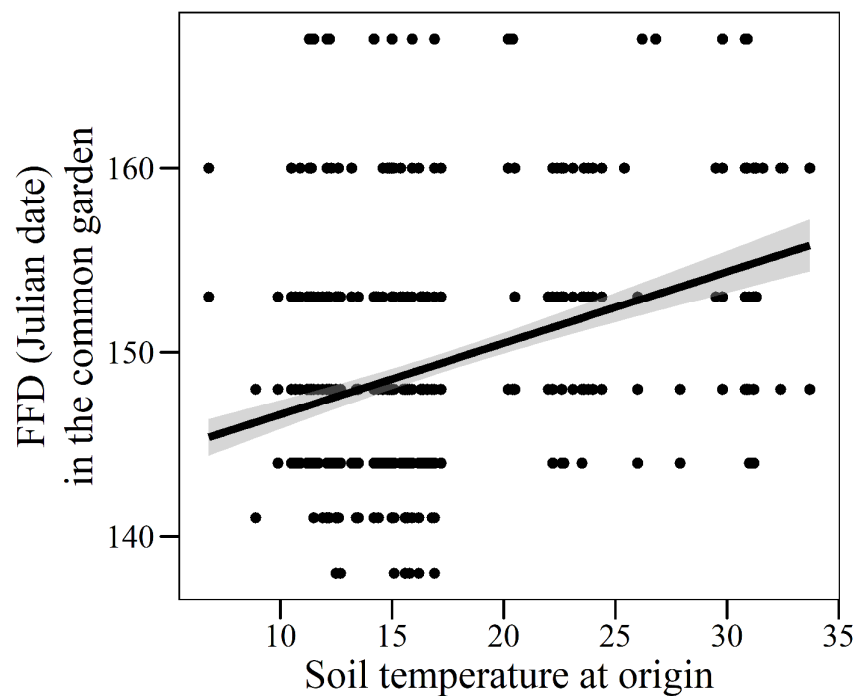


238
239 Phenology in the common garden differed among plants with different mothers (Table
240 3A), and these differences were related to soil temperature at the microsite of origin of the
241 mother plant (Table 3B). As hypothesized, differences in phenology followed a counter-
242 gradient pattern; plants with mothers from colder microsites flowered earlier than plants with
243 mothers from warmer microsites (Fig. 3).

Table 3: Results of linear mixed models on flowering phenology (first flowering date) in the common garden, including effects of (A) mother (random effect), and (B) mother (random effect) and soil temperature at the origin of the mother (fixed effect). The significance of the random effects was tested using a likelihood ratio test (LRT), comparing a model with the random effects to the same model without the random effects.

Model	Predictor variable	LRT χ^2		P
A)	Mother (random)	65.54		<0.001
B)	Mother (random)	25.69		<0.001
		Estimate	SE	t
Soil temperature at origin (fixed)		0.401	0.055	7.24
				<0.001

Figure 3: Effect of soil temperature at the origin of the mother plant on flowering phenology of *Cerastium fontanum* in the common garden in 2017. Phenology was measured as the first flowering date (FFD) converted to Julian date, with higher values representing a later flowering phenology. A linear model fit is shown.



255 DISCUSSION

256 In this study, geothermally induced small-scale variation in soil temperatures was
257 related to both phenotypic and genotypic variation in flowering phenology of the grassland
258 plant *Cerastium fontanum*. Plants at warmer microsites flowered relatively earlier than plants
259 at colder microsites. In contrast, plants grown in a common environment responded to spring
260 warming following a counter-gradient pattern: plants originating from warmer microsites
261 flowered later than those originating from colder microsites. Yet, we were not able to
262 document the corresponding pattern of phenotypic selection on phenology (i.e. stronger
263 selection for early flowering at colder microsites) along the temperature gradient in one study
264 year. From a methodological perspective, our study highlights the suitability of geothermal
265 systems for studying ecological and evolutionary responses to global warming.

266 Our results show a plastic response of *C. fontanum* flowering phenology to natural
267 differences in local soil temperature, with earlier flowering being associated with warmer
268 microsites in two different years. The start of flowering in plants has been reported to advance
269 with increasing temperatures along natural temperature gradients or in response to
270 experimental manipulations (De Frenne et al., 2011; Menzel, Sparks, Estrella, & Roy, 2006;
271 Price & Waser, 1998), and this study highlights that responses to temperature differences also
272 happen over small spatial scales. Previous studies have suggested that the response of
273 flowering time to temperature is mainly due to phenotypic plasticity, which is controlled by
274 plant physiology, and thus can be relatively rapid (De Frenne et al., 2011; Frei et al., 2014).
275 The earlier flowering phenology at warmer microsites along the temperature gradient
276 observed in this study is in agreement with a reported advancement in the start of the growing
277 season in response to warming at a nearby site (Leblans et al., 2017). Previous studies with
278 northern plant species have shown that the physiological control mechanisms of the start of
279 the growing season are to a larger extent driven by heat accumulation (e.g. Growing Degree

280 Days), than by light regime (Bennie, Kubin, Wiltshire, Huntley, & Baxter, 2010). Changes in
281 the persistence of snow cover are also an important driver of flowering time in high latitude
282 and high altitude ecosystems (David W. Inouye & McGuire, 1991). Therefore, the earlier
283 flowering of *C. fontanum* in warmer microsites could be induced both by higher heat
284 accumulation and earlier snowmelt in these microsites.

285 Our results not only provide evidence of phenotypic responses to temperature
286 variation, but also suggest the existence of an evolutionary response to geothermal soil
287 warming. Flowering phenology in a common environment differed among plants with
288 different mothers, and this variation was related to soil temperature at the microsite of origin
289 of the mother plant, suggesting the existence of genetic differentiation in flowering time
290 related to temperature variation. Because we used seeds collected from plants grown in the
291 field, some of the observed variation in flowering phenology in the common garden might
292 potentially be due to non-genetic maternal effects (Galloway, 2005; Rossiter, 1996), rather
293 than due to additive genetic variation. The maternal environment may influence the phenotype
294 of the offspring directly, through seed provisioning, or through plasticity of traits in the
295 maternal plant that influence offspring trait expression (Galloway, 2005). The plants used in
296 our common garden study were grown for two years in a common environment before
297 recording their flowering phenology, which should have decreased, although not eliminated,
298 maternal effects. Moreover, maternal effects are usually most pronounced in early life-history
299 stages (Rossiter, 1996), and thus probably not highly affecting our results. A third reason why
300 maternal effects appear to be less likely to qualitatively have influenced our findings, is that
301 the relationship between temperature at the microsite of origin and flowering phenology in the
302 common garden was in the opposite direction to the pattern observed in the field.

303 In the common garden, plants originating from environments with longer growing
304 seasons (i.e. warmer microsites) started flowering relatively later than plants originating from

environments with shorter growing seasons (i.e. colder microsites). This is in contrast with the pattern in the field, where plants at warmer microsites flowered relatively earlier than plants at colder microsites. These opposite responses have two important implications. First, they suggest that the effect of temperature on phenology in the field is largely plastic. Second, the observed pattern constitutes an example of counter-gradient variation in local adaptation occurring over a very small spatial scale (Conover & Schultz, 1995). The results of our common garden study agree with results obtained over larger-scale temperature gradients. For example, plants originating from northern populations usually flower before plants from southern populations when grown in a common environment (Kollmann & Bañuelos, 2004; Olsson & Ågren, 2002). In cold environments, a later start and a shorter duration of the growing season constrains the period available for growth and fruit maturation, and should select for fast development and early flowering, and result in plants evolving to start development at lower temperatures. The existence of genetically-determined variation in flowering phenology over small spatial scales in our study contributes to the increasing evidence of the importance of microgeographic adaptation (Richardson et al., 2014; Skelly, 2004). For local adaptation to occur, the strength of selection has to exceed the homogenizing effect of gene flow (García-Ramos & Kirkpatrick, 1997). *Cerastium fontanum* reproduces with cross-pollination or selfing. In our study site selfing is likely very common as it has been demonstrated that selfing rates increase with latitude and altitude (Bliss, 1962; Medan et al., 2002). This might lead to a limited gene flow not being strong enough to counteract selection and prevent the evolutionary divergence of plants in cold and warm microsites. Genetic differentiation at such small spatial scales might allow to buffer species against future climatic changes. For instance, plants exposed to locally warm temperatures might supply the adaptive alleles that promote more widespread adaptation to warming temperatures (Richardson et al., 2014), allowing species to persist under future climatic conditions.

Although the results of our common garden experiment suggest the existence of an evolutionary response to geothermal soil warming which should be the consequence of differences in phenotypic selection among cold and warm microsites, we found no evidence of such variation in selection. Contrary to our expectation of natural selection for early flowering being stronger in colder microsites, selection favored early flowering irrespective of soil temperature. The strength and direction of phenotypic selection in natural populations has been shown to vary temporally (Siepielski, DiBattista, & Carlson, 2009), and the conditions favouring stronger selection for early flowering in colder microsites might be present in some years, but absent in others. For example, in years with very cold springs or late spring frosts, where cold temperatures and frost damage could prevent pollination and fruit set in early-flowering plants (D. W. Inouye, 2000), selection for early flowering in colder microsites might be weaker than it would be in years with average spring conditions.

An important implication of the results of our study is that plants are likely to respond to higher temperatures not only plastically, but also genetically, and that observed responses will often be a combination of short-term plastic responses and long-term evolutionary responses. Moreover, our results suggest that phenotypic and genotypic responses to warming might act in opposite directions. In a global warming context, adaptation to climate change may be especially important for the persistence of flowering plant species in high-latitude areas because their limited dispersal ability and the proximity to their range edge may prevent them from shifting their distribution to more favorable climates. Climate exerts strong selective pressure on natural populations, and the results of our study highlight that in order to correctly interpret observed phenotypic responses to past climate change in seasonal environments, as well as to predict responses to future climate change, we need to account for the fact that adaptive evolution and changes in population genetic composition might be as important as the instantaneous plastic responses.

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361 REFERENCES

- Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science*, 294(5541), 321–326. <https://doi.org/10.1126/science.1060701>
- 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 of London B: Biological Sciences*, 279(1743), 3843–3852. <https://doi.org/10.1098/rspb.2012.1051>
- Arft, A. M., Walker, M. D., Gurevitch, J., Alatalo, J. M., Bret-Harte, M. S., Dale, M., ... Wookey, P. A. (1999). Responses of tundra plants to experimental warming: meta-analysis of the International Tundra Experiment. *Ecological Monographs*, 69(4), 491–511. [https://doi.org/10.1890/0012-9615\(1999\)069\[0491:ROTPTE\]2.0.CO;2](https://doi.org/10.1890/0012-9615(1999)069[0491:ROTPTE]2.0.CO;2)
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, 15(4), 365–377. <https://doi.org/10.1111/j.1461-0248.2011.01736.x>
- Bennie, J., Kubin, E., Wiltshire, A., Huntley, B., & Baxter, R. (2010). Predicting spatial and temporal patterns of bud-burst and spring frost risk in north-west Europe: the implications of local adaptation to climate. *Global Change Biology*, 16(5), 1503–1514. <https://doi.org/10.1111/j.1365-2486.2009.02095.x>
- Bliss, L. C. (1962). Adaptations of arctic and alpine plants to environmental conditions. *Arctic*, 15(2), 117–144.

- Chapin III, F. S. C., Matson, P. A., & Vitousek, P. (2011). *Principles of Terrestrial Ecosystem Ecology* (2nd ed.). New York: Springer-Verlag. Retrieved from [//www.springer.com/la/book/9781441995032](http://www.springer.com/la/book/9781441995032)
- Cleland, E. E., Chuine, I., Menzel, A., Mooney, H. A., & Schwartz, M. D. (2007). Shifting plant phenology in response to global change. *Trends in Ecology & Evolution*, 22(7), 357–365.
- Cleland, Elsa E., Chiariello, N. R., Loarie, S. R., Mooney, H. A., & Field, C. B. (2006). Diverse responses of phenology to global changes in a grassland ecosystem. *Proceedings of the National Academy of Sciences*, 103(37), 13740–13744. <https://doi.org/10.1073/pnas.0600815103>
- Conover, D. O., & Schultz, E. T. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology & Evolution*, 10(6), 248–252. [https://doi.org/10.1016/S0169-5347\(00\)89081-3](https://doi.org/10.1016/S0169-5347(00)89081-3)
- De Frenne, P., Brunet, J., Shevtsova, A., Kolb, A., Graae, B. J., Chabrierie, O., ... Verheyen, K. (2011). Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Global Change Biology*, 17(10), 3240–3253. <https://doi.org/10.1111/j.1365-2486.2011.02449.x>
- De Frenne, P., Graae, B. J., Rodríguez-Sánchez, F., Kolb, A., Chabrierie, O., Decocq, G., ... Verheyen, K. (2013). Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology*, 101(3), 784–795. <https://doi.org/10.1111/1365-2745.12074>
- 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(4), 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(1), 123–139. <https://doi.org/10.1111/eva.12112>
- Frei, E. R., Ghazoul, J., Matter, P., Heggli, M., & Pluess, A. R. (2014). Plant population differentiation and climate change: responses of grassland species along an elevational

- gradient. *Global Change Biology*, 20(2), 441–455. <https://doi.org/10.1111/gcb.12403>
- Galloway, L. F. (2005). Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist*, 166(1), 93–100. <https://doi.org/10.1111/j.1469-8137.2004.01314.x>
- García-Ramos, G., & Kirkpatrick, M. (1997). Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, 51(1), 21–28. <https://doi.org/10.1111/j.1558-5646.1997.tb02384.x>
- Gudmundsdottir, R., Gislason, G. M., Pálsson, S., Olafsson, J. S., Schomacker, A., Friberg, N., ... Moss, B. (2011). Effects of temperature regime on primary producers in Icelandic geothermal streams. *Aquatic Botany*, 95(4), 278–286. <https://doi.org/10.1016/j.aquabot.2011.08.003>
- Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, 470(7335), 479–485. <https://doi.org/10.1038/nature09670>
- Ibáñez, I., Primack, R. B., Miller-Rushing, A. J., Ellwood, E., Higuchi, H., Lee, S. D., ... Silander, J. A. (2010). Forecasting phenology under global warming. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 365(1555), 3247–3260. <https://doi.org/10.1098/rstb.2010.0120>
- Inouye, D. W. (2000). The ecological and evolutionary significance of frost in the context of climate change. *Ecology Letters*, 3(5), 457–463. <https://doi.org/10.1046/j.1461-0248.2000.00165.x>
- Inouye, David W., & McGuire, A. D. (1991). Effects of snowpack on timing and abundance of flowering in *Delphinium nelsonii* (Ranunculaceae): implications for climate change. *American Journal of Botany*, 78(7), 997–1001. <https://doi.org/10.2307/2445179>
- IPCC. (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.
- Kollmann, J., & Bañuelos, M. J. (2004). Latitudinal trends in growth and phenology of the

- invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Diversity and Distributions*, 10, 377–385.
- Körner, C. (2007). The use of ‘altitude’ in ecological research. *Trends in Ecology & Evolution*, 22(11), 569–574. <https://doi.org/10.1016/j.tree.2007.09.006>
- Kristinsson, H. G. (1998). *A guide to the flowering plants and ferns of Iceland* (2nd Revised edition). Reykjavík: Mal Og Menning, Iceland.
- Lavergne, S., Mouquet, N., Thuiller, W., & Ronce, O. (2010). Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. *Annual Review of Ecology, Evolution, and Systematics*, 41(1), 321–350. <https://doi.org/10.1146/annurev-ecolsys-102209-144628>
- Leblans, N. I. W., Sigurdsson, B. D., Vicca, S., Fu, Y., Penuelas, J., & Janssens, I. A. (2017). Phenological responses of Icelandic subarctic grasslands to short-term and long-term natural soil warming. *Global Change Biology*, 23(11), 4932–4945. <https://doi.org/10.1111/gcb.13749>
- Linderholm, H. W. (2006). Growing season changes in the last century. *Agricultural and Forest Meteorology*, 137(1), 1–14. <https://doi.org/10.1016/j.agrformet.2006.03.006>
- Medan, D., Montaldo, N. H., Devoto, M., Maniese, A., Vasellati, V., Roitman, G. G., & Bartoloni, N. H. (2002). Plant-pollinator relationships at two altitudes in the Andes of Mendoza, Argentina. *Arctic, Antarctic, and Alpine Research*, 34(3), 233–241. <https://doi.org/10.1080/15230430.2002.12003490>
- Menzel, A., Sparks, T. H., Estrella, N., & Roy, D. B. (2006). Altered geographic and temporal variability in phenology in response to climate change. *Global Ecology and Biogeography*, 15(5), 498–504. <https://doi.org/10.1111/j.1466-822X.2006.00247.x>
- Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications*, 7(1), 1–14. <https://doi.org/10.1111/eva.12137>
- O’Gorman, E. J., Benstead, J. P., Cross, W. F., Friberg, N., Hood, J. M., Johnson, P. W., ... Woodward, G. (2014). Climate change and geothermal ecosystems: natural laboratories, sentinel systems, and future refugia. *Global Change Biology*, 20(11),

- 3291–3299. <https://doi.org/10.1111/gcb.12602>
- Olsson, K., & Ågren, J. (2002). Latitudinal population differentiation in phenology, life history and flower morphology in the perennial herb *Lythrum salicaria*. *Journal of Evolutionary Biology*, 15(6), 983–996. <https://doi.org/10.1046/j.1420-9101.2002.00457.x>
- Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37(1), 637–669. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110100>
- Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421(6918), 37–42. <https://doi.org/10.1038/nature01286>
- Perron, N. S. (2017). *Warming responses of two native Icelandic species, Ranunculus acris and Thymus praecox ssp. arcticus in geothermal areas* (MsC Thesis). Retrieved from <https://skemman.is/handle/1946/27751>
- Price, M. V., & Waser, N. M. (1998). Effects of experimental warming on plant reproductive phenology in a subalpine meadow. *Ecology*, 79(4), 1261–1271. [https://doi.org/10.1890/0012-9658\(1998\)079\[1261:EOEWOP\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1998)079[1261:EOEWOP]2.0.CO;2)
- Richardson, J. L., Urban, M. C., Bolnick, D. I., & Skelly, D. K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology & Evolution*, 29(3), 165–176. <https://doi.org/10.1016/j.tree.2014.01.002>
- Robinson, S. I., McLaughlin, Ó. B., Marteinsdóttir, B., & O’Gorman, E. J. (2018). Soil temperature effects on the structure and diversity of plant and invertebrate communities in a natural warming experiment. *Journal of Animal Ecology*, 634–646. [https://doi.org/10.1111/1365-2656.12798@10.1111/\(ISSN\)1365-2656.Global_Change_EcologyJANE2018](https://doi.org/10.1111/1365-2656.12798@10.1111/(ISSN)1365-2656.Global_Change_EcologyJANE2018)
- Rossiter, M. (1996). Incidence and consequences of inherited environmental effects. *Annual Review of Ecology and Systematics*, 27(1), 451–476. <https://doi.org/10.1146/annurev.ecolsys.27.1.451>
- Saemundsson, K. (1967). Vulkanismus und tektonik des Hengill-Gebietes in Südwest-Island. *Acta Naturalia Islandica*, 2, 1–105.

- Saemundsson, K. (1992). Geology of the Thingvallavatn Area. *Oikos*, 64(1/2), 40–68.
<https://doi.org/10.2307/3545042>
- Saemundsson, K. (1995). Geological map of the Hengill area 1:50,000. Orkustofnun, Reykjavík.
- Siepielski, A. M., DiBattista, J. D., & Carlson, S. M. (2009). It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecology Letters*, 12(11), 1261–1276.
<https://doi.org/10.1111/j.1461-0248.2009.01381.x>
- Skelly, D. K. (2004). Microgeographic countergradient variation in the wood frog, *Rana sylvatica*. *Evolution*, 58(1), 160–165. <https://doi.org/10.1111/j.0014-3820.2004.tb01582.x>
- Toftegaard, T., Posledovich, D., Navarro-Cano, J. A., Wiklund, C., Gotthard, K., & Ehrlén, J. (2016). Variation in plant thermal reaction norms along a latitudinal gradient – more than adaptation to season length. *Oikos*, 125(5), 622–628.
<https://doi.org/10.1111/oik.02323>
- Visser, M. E. (2008). Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society of London B: Biological Sciences*, 275(1635), 649–659. <https://doi.org/10.1098/rspb.2007.0997>
- Wolkovich, E. M., Cook, B. I., Allen, J. M., Crimmins, T. M., Betancourt, J. L., Travers, S. E., ... Cleland, E. E. (2012). Warming experiments underpredict plant phenological responses to climate change. *Nature*, 485(7399), 494–497.
<https://doi.org/10.1038/nature11014>
- Zakharova, O. K., & Spichak, V. V. (2012). Geothermal fields of Hengill Volcano, Iceland. *Journal of Volcanology and Seismology*, 6(1), 1–14.
<https://doi.org/10.1134/S074204631201006X>