SPECIAL ISSUE ARTICLE

Botany
American Journal of

Selection against early flowering in geothermally heated soils is associated with pollen but not prey availability in a carnivorous plant

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Abstract

Premise: In high-latitude environments, plastic responses of phenology to increasing spring temperatures allow plants to extend growing seasons while avoiding late frosts. However, evolved plasticity might become maladaptive if climatic conditions change and spring temperatures no longer provide reliable cues for conditions important for fitness. Maladaptative phenological responses might be related to both abiotic factors and mismatches with interacting species. When mismatches arise, we expect selection to favor changes in phenology.

Methods: We combined observations along a soil temperature gradient in a geothermally heated area with pollen and prey supplementation experiments and examined how phenotypic selection on flowering time in the carnivorous plant *Pinguicula vulgaris* depends on soil temperature, and pollen and prey availability.

Results: Flowering advanced and fitness decreased with increasing soil temperature. However, in pollen-supplemented plants, fitness instead increased with soil temperature. In heated soils, there was selection favoring later flowering, while earlier flowering was favored in unheated soils. This pattern remained also after artificially increasing pollen and prey availability.

Conclusions: Plant–pollinator mismatches can be an important reason why evolved plastic responses of flowering time to increasing spring temperatures become maladaptive under novel environmental conditions, and why there is selection to delay flowering. In our study, selection for later flowering remained after artificially increasing pollen availability, suggesting that abiotic factors also contribute to the observed selection. Identifying the factors that make evolved phenological responses maladaptive under novel conditions is fundamental for understanding and predicting evolutionary responses to climate warming.

KEYWORDS

aster models, geothermal ecosystems, local adaptation, microclimate, phenotypic plasticity, phenotypic selection, *Pinguicula*, plant phenology, pollination, temperature variation

Phenotypic plasticity—the ability of a genotype to produce different phenotypes in response to different environmental conditions—might be adaptive, maladaptive, or neutral with respect to an individual's fitness (Ghalambor et al., 2007). Given genetic variation in phenotypic plasticity, natural selection will favor the pattern of plasticity that represents the most beneficial response to the range of environmental conditions encountered by an organism during its lifetime (Schlichting, 1986; Gavrilets and Scheiner, 1993; de Jong, 2005). Environmental changes, such as climate

This article is part of the *AJB* Special Issue "Approaches to the Study of Quantitative Fitness-Related Traits."

warming, might expose organisms to conditions that largely fall outside the range of conditions under which plasticity evolved (Berteaux et al., 2004; Visser, 2008). In these cases, plastic responses might become maladaptive, i.e., causing traits to shift in a direction that leads to a decrease in fitness (Ghalambor et al., 2007; Scheepens and Stöcklin, 2013; Radersma et al., 2020; Stamp and Hadfield, 2020). In environments that have recently changed, and where patterns of plasticity evolved under previous conditions reduce fitness, we thus expect selection to favor changes that restore adaptive plasticity (Grether, 2005; Nussey et al., 2005; Ghalambor et al., 2007; Anderson et al., 2012; Svensson et al., 2020).

Identifying the conditions that make plastic responses adaptive vs. maladaptive is fundamental to understand the evolution of plasticity. Several factors can potentially make evolved patterns of plasticity become maladaptive when the environment changes (Wadgymar et al., 2017). In seasonal environments, abiotic conditions such as the relationship between spring temperature and season length are of crucial importance to many organisms, and changes in these relationships might make evolved plastic responses of development to temperatures malaptative (Springate and Kover, 2014; Isaac-Renton et al., 2018). Changes in interactions among species, e.g., caused by changes in timing relative to prey resources, mutualists, consumers and competitors, might also be a major reason why plasticity becomes maladaptive in novel environments. For example, plastic responses of egg-laying dates in birds to increases in spring temperature might sometimes result in mismatches with their insect food resources (Both et al., 2006; Nussey et al., 2005; Visser et al., 1998). Differences in the responses to warming between species involved in interactions are common (Kharouba et al., 2018; Renner and Zohner, 2018; Kudo and Cooper, 2019), and such differences can arise because interacting species respond differently to the same cues or because they respond to different cues. For example, pollinator emergence might be triggered by cues other than flowering onset (Forrest and Thomson, 2011; Iler et al., 2013). Moreover, mismatches can arise when interacting species experience different degrees of warming, either because they use niches that differ in microclimate, or because warming is spatially heterogeneous and interaction partners sample the environment over different spatial scales.

In high-latitude seasonal environments, where growing seasons are on average short and variable in length, plasticity in the timing of response of key life-history events to increasing spring temperatures is critical for performance. For example, by matching their flowering time to spring temperatures, plants can benefit from a long growing season, while at the same time avoiding exposure to unfavorable conditions (Inouye, 2000; Lenz et al., 2013; Ehrlén, 2015). Thermal plasticity in reproductive timing is considered to be adaptive in high-latitude environments because spring temperature serves as a reliable cue for future growth conditions, including the probability of

spring frosts, as well as for the availability of pollinating insects. However, if these relationships change, evolved patterns of plasticity might become maladaptive. For example, in geothermally heated areas, soil temperature might no longer constitute a reliable cue for air temperatures and subsequent growth conditions (O'Gorman et al., 2014; Valdés et al., 2019). Evolved responses to temperature in these soils might therefore lead to plants initiating reproductive development too early and experiencing an increased risk of frost damage. In addition, plants on heated soils might be poorly synchronized with their pollinators because when warming is very local, sessile organisms such as plants experience much larger increases in mean temperatures than mobile insects, which after emergence in spring are strongly influenced by air temperature. As a result of such mismatches, plastic responses to novel conditions might sometimes be too extreme and "overshoot" the optimal local phenotype (cf. Radersma et al., 2020; Stamp and Hadfield, 2020; Svensson et al., 2020). In such cases, we therefore should expect that selection favors a lower responsiveness of plant phenology to increasing soil temperatures, and a later flowering time (Grether, 2005).

Geothermal ecosystems often consist of small-scale mosaics of areas heated by geothermal steam and unheated areas, with a large variation in temperature and snowmelt date over small spatial scales, but little variation in other abiotic factors (O'Gorman et al., 2014; Valdés et al., 2019). These natural thermal gradients thus avoid many of the limitations of larger-scale temperature gradients (e.g., latitudinal or altitudinal, De Frenne et al., 2013; Ensing and Eckert, 2019), where other factors might covary with temperature. In this study, we used such a "natural heating experiment" and combined field observations of the perennial, carnivorous plant *Pinguicula vulgaris* (Lentibulariaceae) along a geothermal soil warming gradient in a subarctic Icelandic grassland, with experimental manipulations of pollen and prey availability. We used this setup to investigate how flowering time, fitness, and phenotypic selection on flowering time vary with soil temperature and to what extent selection is related to the availability of pollen and prey. Due to the short and variable growing seasons in subarctic environments, we expected plants in unheated areas to have evolved strong plastic responses of flowering time to temperature increases in spring. We also hypothesized that these responses become maladaptive in heated soils, either because soil temperature is not a reliable cue for spring advancement, or because early flowering leads to mismatches with pollinators and prey. In heated soils, we thus expected selection for later flowering, acting to restore interactions with pollinators and prey, for example, by an increased reliance on day length as a cue. Based on this, we made five predictions: (1) Plants respond plastically to small-scale variation in soil temperatures and flower earlier at higher soil temperatures. (2) This phenological response becomes maladaptive in heated soils and affects plant fitness negatively. (3) Pollen and prey supplementation mitigate

the negative effects of high soil temperatures on fitness. (4) Selection on flowering phenology acts to decrease the negative effects of maladaptive phenological responses in heated areas by favoring later flowering. (5) Pollen and prey supplementation weaken selection for later flowering in heated areas by mitigating negative effects of mismatches with pollinators and prey.

MATERIALS AND METHODS

Study site and system

The study was conducted during the summer of 2020 in a subarctic geothermal area in Ölfus municipality in southwestern Iceland (64°03'N; 21°18'W, ~360 m a.s.l.), belonging to the Hengill volcanic system (Zakharova and Spichak, 2012). In Iceland, high-temperature geothermal areas are located near tectonic plate boundaries, where the groundwater is heated by magma chambers found at shallow depth. Accumulations of heated water create high-pressure areas that warm the soil above, with soil temperatures that can exceed 50°C above ambient in the Hengill area (Perron, 2017). Soil heating decreases with distance to geothermal hotspots, forming a mosaic of soil temperature over the geothermal system (Sigurdsson et al., 2016). The study area consists of approximately 1 km² with gradients of soil temperature ranging from no geothermal heating to more than 20°C above ambient temperature over short distances, with small differences in other environmental factors, such as soil chemistry, elevation, and vegetation type (O'Gorman et al., 2014; Robinson et al., 2018). Warm soils have usually been geothermally heated for relatively long periods, probably well over 50 years (Saemundsson, 1967). However, small changes in the intensity of heating might sometimes occur over shorter time scales (B. Marteinsdottir, personal observations). Over long periods, hundreds to thousands of years, the spatial pattern of geothermal soil heating is dynamic, e.g., due to changes in geothermal activity caused by earthquakes (Barbier, 2002). Differences in the phenology of other plant species along the natural geothermal soil heating gradients have been documented in previous studies (Perron, 2017; Valdés et al., 2019).

The study species, the common butterwort (*Pinguicula vulgaris*), is a carnivorous, perennial herb with a circumboreal distribution (Heslop-Harrison, 2004). It has a small, annual root system, and individuals survive the winter as subterranean buds below the soil surface. These overwintering buds contain leaf and floral primordia that are formed in the season before the leaves and flowers develop (Worley and Harder, 1999). New leaves and roots develop in late May to early June. Survival of established plants is high, with an estimated 92% yearly survival of established individuals in another subarctic population (Svensson et al., 1993). Vegetative propagation by subsidiary subterranean buds is common (Worley and Harder, 1999). The leaves are curled up at the margins and are arranged in a basal rosette. Along with the basal part of the flowering stems, they excrete mucilage that attracts and traps small insects, which are then digested and absorbed (Hanslin and Karlsson, 1996). Insect prey has been reported to provide 26 to 40% of the annual nitrogen requirements of the plant, with Collembola and Nematocera being the most common prey types (Karlsson et al., 1994). Plants need to reach a certain threshold size to flower, but skip flowering in some years after reaching this threshold (Méndez and Karlsson, 2004; Worley and Harder, 1996). The leafless flowering stems (ca. 5–10 cm high) bear single purple flowers. Most plants produce a single flowering stem, although up to three stems is common, and the observed maximum is six. Flowering occurs from June to August in the study area. Fruits are capsules containing about 100–350 small seeds. Flowers are able to self-pollinate autonomously, but seed set has been reported to be lower when insect pollinators are excluded (Molau, 1993). Other studies have indicated that reproduction is limited also by resource availability (Thorén et al., 1996; Worley and Harder, 1996).

Data collection

In late May to early June 2020, 450 individuals of *P. vulgaris*, distributed along the entire temperature gradient in the study area, were marked. Each individual was marked by a nail with a numbered plastic tag and its position recorded on a map. At the time of marking, and again in July and August, soil temperature at a depth of 10 cm in the immediate vicinity (<2 cm) of each plant was measured with a digital multipurpose thermometer. Individuals were marked before the development of flower buds to avoid bias with regards to flowering time and to be able to follow them from the start of flower development. As a result, a large proportion of marked individuals did not flower, and we performed our analyses using the 287 individuals that flowered.

To describe flowering phenology, floral development was monitored every week. At each recording, each plant was assigned to one of nine floral development stages (1 = only vegetative growth, the plant only has leaves, 2 = buds just starting to form, very small, close to leaves; 3 = largest bud of medium size; 4 = largest bud large but still completely closed; 5 = largest bud starting to open; 6 = at least one open flower; 7 = all flowers opened, none yet wilted; 8 = at least one wilted flower; 9 = all flowers wilted). For each plant, we used the mean of the date of the last recording with buds and the date of the first recording with flowers as a first estimate of first flowering date (FFD). Developmental stages for plants varied on these dates, i.e., at the last recording with buds, a plant could be on stages 1–5 and at the first recording with flowers on stages 6–9. To distribute estimates of FFD evenly across recording intervals and to estimate FFD for each individual as accurately as possible, we used information about the development stage

at the last recording with buds, and at the first recording with flowers. We assigned each plant to the most likely FFD by adding one term to the first estimate of FFD that was based on the stage at the last recording with buds (+0.2 × recording interval for stages 1 and 2, 0 for stage 3, and –0.2 × recording interval for stages 4 and 5) and a second term based on the stage at the first recording with flowers (+0.2 × recording interval for stages 6 and 7, and –0.2 × recording interval for stages 8 and 9). For example, if the first estimate of FFD was 160 (8 June), the recording interval 7 days, the stage at the last recording with buds was 3, and the stage at the first recording with flowers was 6, the most likely FFD would be 160 + 0 + 1.4 = 161.4.

For each individual, the number of flowering stems was counted, and the longest diameter of the rosette and the diameter perpendicular to that were measured at the time when at least half of the flowers had opened. In late August, the number of mature intact fruits was counted in the field. All fruits were collected and brought to the lab, where the seeds in each fruit were counted. We used the total number of seeds of an individual as a measure of plant fitness. Fitness data was available for 274 individuals.

To examine whether reproduction was limited by the availability of insect pollinators and prey, we carried out experimental manipulations of both of these interactions and assessed the effects on fitness and phenotypic selection. Flowering individuals were randomly assigned to one of four treatments: pollen supplementation (68 individuals), prey supplementation (69 individuals), pollen and prey supplementation (71 individuals), and control (79 individuals). Both treatments were stratified to assure that they were equally distributed among soil temperatures and flowering times. The pollen supplementation treatment consisted of hand-pollinating all open flowers with a brush, using a mix of pollen from multiple individuals in the near vicinity of the focal individual. Treatments were applied to each plant approximately twice per week, from the day when the first flower opened on the individual until the day when all flowers had wilted. The pollen supplementation treatment thus corresponded to increased levels of pollination compared with control plants, but not necessarily to the maximum possible level of pollination. Prey supplementation was done by adding three *Simulium vittatum* flies per week during the 7 weeks when individuals in the study area typically capture prey, i.e., from late June when leaves have developed until mid-August when leaves start to senesce. This treatment corresponded to the highest level of prey observed in unmanipulated individuals in the study area (approximately 25% of the individuals that had captured prey had three or more prey of corresponding size). The flies were collected in the field using an aspirator device, either directly from the heads of field workers (as the flies are very obtrusive during peak season), or first caught in a large sweep net. Due to low numbers of wild *Simulium*, particularly during the first phase of the experiment, we also used similar-sized *Drosophila melanogaster* flies as a supplement when the availability of *Simulium* was low.

Drosophila were raised in tubes with food at the bottom, and mature flies were transferred to separate tubes and killed by freezing. They were kept frozen until fed to *Pinguicula*. To mimic the movement of live prey, they were joggled around slightly before being placed under the curved leaf margins using tweezers. This placement was done to reduce cleptoparasitism (although no ants have been recorded in the study area), to avoid that the prey was washed away by rain, and to maximize the contact with the digestive leaf surface (Hanslin and Karlsson, 1996).

Statistical analyses

To assess whether plants flower earlier at higher soil temperatures (Prediction 1), we fitted a linear model of FFD on soil temperature. We also included a quadratic term for soil temperature to test for a nonlinear relationship. To evaluate the effects of soil temperature, FFD, and pollen and prey supplementation on fitness and phenotypic selection on flowering time, we used an aster model because a large proportion of individuals (ca. 50%) produced zero seeds (Geyer et al., 2007; Shaw et al., 2008; Shaw and Geyer, 2010). Aster models account for the nonconformity of fitness to common statistical distributions, appropriately modeling the distribution of each fitness component, and accounting for the dependency of each component on earlier stages in a joint analysis that produces valid standard errors for mean fitness. Our aster model incorporated three components of fitness: fruit production (i.e., if the plant produced any fruits, modeled as a 0/1 Bernouilli variable), fruit number (modeled as a zero-truncated negative binomial, because fruit number cannot be zero if fruit production = 1), and seed number (modeled as a Poisson variable). As predictors, the model included soil temperature, FFD, and exposure to two crossed experimental treatments, "pollen" and "prey", telling if the plant was supplemented with pollen (supplemented = 1, not supplemented = 0) and prey (supplemented = 1, not supplemented = 0), respectively. Models included all possible interactions between soil temperature, FFD, pollen and prey. Log-transformed rosette area (in square centimeters, calculated using the formula for the area of an ellipse) was included as a covariate to account for differences in plant condition. To evaluate whether pollen and prey supplementation altered the relationship between fitness and soil temperature (Predictions 2 and 3), we examined the effects of the interactions soil temperature × pollen, and soil temperature × prey. To evaluate whether selection on phenology was temperature-dependent (Prediction 4), we examined the effect of the interaction soil temperature × FFD. Lastly, to evaluate whether pollen and prey supplementation reduced the effect of temperature on selection on phenology (Prediction 5), we examined the effects of the interactions soil temperature × FFD × pollen, and soil temperature × FFD × prey. We also tested whether the

effects of pollen and prey supplementation were interactive by including the interaction pollen × prey and the interactions soil temperature × pollen × prey, FFD × pollen × prey, and soil temperature × FFD × pollen × prey. We mean-centered soil temperature, FFD, and rosette area to help the model converge. For estimating the effects of each predictor on fitness, each predictor was fit at the level of seed number in the model (Shaw et al., 2008). We determined the significance of main effects and interactions by comparing submodels without the term of interest to the full model using likelihood ratio tests (LRTs)s.

All statistical analyses were carried out in R version 4.1.0 (R Core Team, 2021) using the package aster (Geyer, 2019).

RESULTS

Soil temperature ranged from 8.6 to 26.6°C (mean ± SD = 13.5 ± 3.6), first flowering date of individuals from May 29 to July 18 (mean ± SD = June 27 ± 9.0 days), and fitness of plant individuals from 0 to 551 seeds (mean ± SD = 110.7 ± 136.1). Most plant individuals (83.5%, $N = 284$) produced only a single flower, and soil temperature did not influence the incidence of multiple flowers (logistic regression of individuals having a single flower vs. multiple flowers on soil temperature: $\beta = -0.015$, $P = 0.748$).

Plants flowered earlier at higher soil temperatures (Prediction 1), and the effect of soil temperature was nonlinear, slightly decreasing at higher temperatures (Figure 1). The model predicted that plants growing on the warmest soils, initiated flowering on average 36 days earlier than plants on the coldest soils.
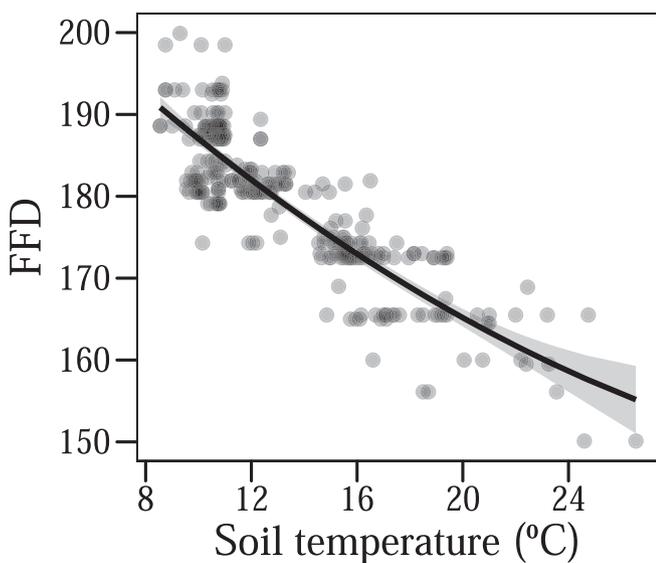
Fitness decreased with soil temperature in plants that were not supplemented with pollen (Prediction 2). This negative relationship between soil temperature and fitness was reversed by pollen supplementation; i.e., fitness increased with soil temperature in plants that were supplemented with pollen (Prediction 3, significant effect of the interaction soil temperature × pollen; Table 1, Figure 2). In plants not supplemented with pollen, the aster model predicted that individuals on the coldest soils (5th percentile) produced on average 172.4 seeds per plant, while individuals on the warmest soils (95th percentile) produced on average 109.3 seeds. In plants supplemented with pollen, it predicted that individuals on the coldest soils produced on average 101.4 seeds, while individuals on the warmest soils produced on average 833.6 seeds. Prey supplementation did not have any effect on the relationship between fitness and soil temperature, and there were no effects of the interaction between pollen and prey supplementation (Table 1).

Selection on phenology was influenced by soil temperature; early flowering was relatively more favored on colder soils, while late flowering was more favored on warmer soils (Prediction 4, significant effect of the interaction soil temperature × FFD; Table 1, Figure 3). The aster model

TABLE 1 Summary of aster model testing for effects of soil temperature, first flowering date (FFD), and pollen and prey supplementation on fitness. The model was fitted with all possible interactions between soil temperature, FFD, pollen supplementation and prey supplementation. Log-transformed rosette area was included as a covariate. The model incorporated three components of fitness: fruit production (i.e., if the plant produced any fruits, modeled as a 0/1 Bernouilli variable), fruit number (modeled as a zero-truncated negative binomial, because fruit number cannot be zero if fruit production = 1), and seed number (modeled as a Poisson variable). Results of likelihood ratio tests for significance of each model term are shown, and boldface indicates significant terms.

Predictor variable	Test deviance	P
Soil temperature	0.032	0.859
FFD	0.445	0.505
Pollen	0.003	0.955
Preys	0.499	0.480
Rosette area	0.314	0.575
Soil temperature × Pollen	**7.360**	**0.007**
Soil temperature × Prey	0.052	0.819
Soil temperature × FFD	**31.767**	**<0.001**
FFD × Pollen	2.910	0.088
FFD × Prey	0.103	0.748
Soil temperature × FFD × Pollen	1.753	0.186
Soil temperature × FFD × Prey	0.481	0.488
Pollen × Prey	0.835	0.361
Soil temperature × Pollen × Prey	0.228	0.633
FFD × Pollen × Prey	0.014	0.905
Soil temperature × FFD × Pollen × Prey	0.002	0.964

FIGURE 1 Relationship between first flowering date (FFD) of *Pinguicula vulgaris* individuals and local soil temperature. Points represent actual observations. The line shows the predicted effect and confidence intervals from a quadratic model of FFD on soil temperature. Linear slope of FFD on soil temperature = –3.376, $t = -6.51$, $P < 0.001$, quadratic slope of FFD on soil temperature = 0.040, $t = 2.36$, $P = 0.019$.
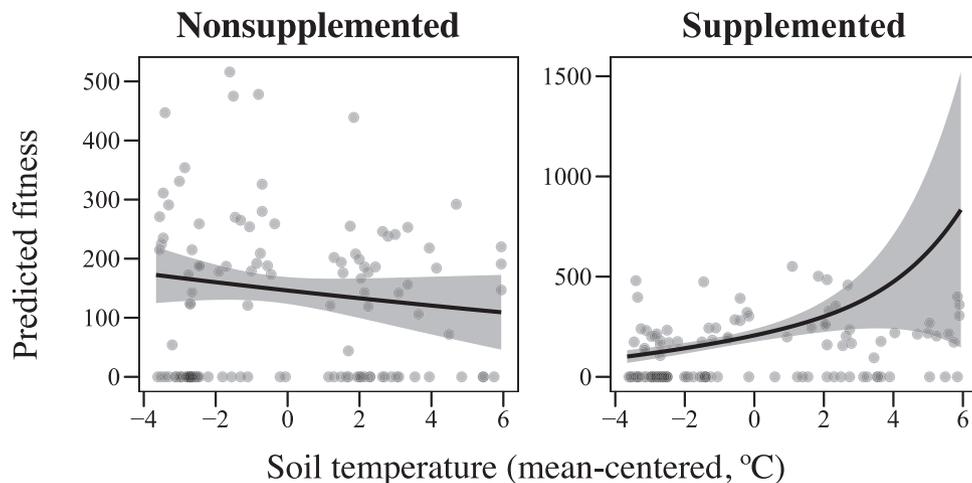
FIGURE 2 Effect of pollen supplementation on the relationship between fitness and soil temperature in *Pinguicula vulgaris*. The left panel shows the relationship for individuals experiencing ambient levels of pollination, and the right panel shows the relationship for individuals that had received a pollen-supplementation treatment. Points represent actual observations. The lines show predicted effects for nonsupplemented and supplemented plants on fitness from the aster model. Predictions were calculated for soil temperatures ranging from the 5th percentile to the 95th percentile to avoid making predictions based on very few observations
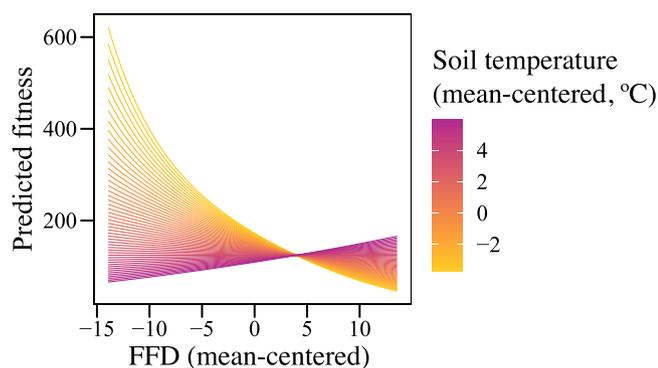

FIGURE 3 Effect of soil temperature on the relationship between fitness and FFD in *Pinguicula vulgaris*. Lines of different colors represent relationships at different soil temperatures, from cold (yellow) to warm (purple) and show predicted effects on fitness from the aster model. Predictions were calculated for soil temperatures ranging from the 5th percentile to the 95th percentile to avoid making predictions based on very few observations.

predicted that selection favored earlier flowering at soil temperatures up to 16.9°C, which is about 7°C warmer than at unheated soils (median of the first quartile of plant soil temperatures = 10.1°C), but later flowering at temperatures higher than this.

Pollen and prey supplementation did not affect the relationship between temperature and selection on phenology, and there was no effect of the interaction between pollen and prey supplementation (Prediction 5, Table 1).

DISCUSSION

In this study, we investigated whether there is phenotypic selection to modify flowering phenology of a carnivorous plant in geothermally heated soils and to what extent such selection is driven by the availability of pollen and prey. In agreement with our hypotheses, we found that individuals started flowering earlier but had lower fitness at higher soil temperatures. In individuals that were pollen-supplemented, fitness instead increased with soil temperature, suggesting that pollinators contributed to the decrease in fitness with earlier flowering in heated soils. Contrary to our hypothesis, prey supplementation did not affect the relationship between fitness and soil temperature. Importantly, in agreement with our hypothesis, we found evidence of temperature-dependent phenotypic selection, favoring later flowering in heated soils. This temperature-dependent selection could not be linked to a specific biotic interaction. Taken together, our results suggest that responses of flowering time to soil temperatures in spring are maladaptive on heated soils and that these maladaptive responses are partly driven by mismatches with pollinators. However, the fact that selection favored later flowering also in plants where pollen availability was artificially restored suggests that other factors also play a role. Our findings are important because they document selection acting to modify phenological responses to soil warming and identify pollen availability as an agent of temperature-dependent selection.

Pinguicula vulgaris individuals started to flower significantly earlier in warmer soils, suggesting that they respond plastically to small-scale variations in soil temperature. A plastic response in the same direction was found for another perennial herb, *Cerastium fontanum*, in the same area (Valdés et al., 2019). The effect of soil temperature on flowering time in *C. fontanum* was weaker than in *P. vulgaris* (0.19 vs. 3.38 days earlier for each °C of increase in temperature, respectively). A possible explanation for this difference is that pollinating and prey insects present in the area have a relatively high temperature sensitivity and that individuals of *P. vulgaris*,

which depend on insects for pollination and as prey, have been selected to match their phenology to insects, whereas this has been less important for *C. fontanum*, which is largely self-pollinating and not carnivorous.

We also found, in agreement with our hypothesis, that fitness in unmanipulated individuals decreased with soil temperature. This is consistent with the assumption that plastic responses of phenology to temperature increases in spring have evolved under conditions without geothermal heating, i.e., with short and variable growing seasons where soil temperature provides a reliable cue for air temperature, spring advancement and pollinator availability. In heated soils, these responses might become maladaptive because growing seasons are longer and an early start of reproduction is less beneficial (Leblans et al., 2017), and because soil temperature is a less reliable cue for air temperature and subsequent conditions.

If responses of flowering phenology to temperature that have evolved under unheated conditions become maladaptive on geothermally heated soils because of the reasons discussed above, then we expect selection in these areas to favor an altered response of flowering phenology to temperature increase in spring and a later flowering at a given temperature. Given that there is sufficient genetic variation in phenology responses, plants in heated areas should eventually become adapted to the new conditions. That we found ongoing selection for later flowering in heated soils in *P. vulgaris* thus suggests that plants have not yet fully adapted to the new conditions. A previous study found a similar pattern for *Cerastium fontanum* on heated soils in the same study area, and the reasons for this pattern are likely similar to those suggested for that species (Valdés et al., 2019). Given that the temperature-dependent selection that we observed during our study is representative for conditions over longer periods, there are two possible explanations for why populations have still not fully adapted to conditions in heated areas. First, given the high survival of established plants and the prevalence of vegetative propagation in *P. vulgaris* (Svensson et al., 1993; Worley and Harder, 1999), several decades of soil heating might be insufficient for plants to evolve to the optimum. Second, differences in soil temperature frequently occur over short distances. Pollinator movements and seed dispersal might therefore result in non-negligible gene flow that prevents plants from fully adapting to local soil temperatures, and the observed selection might represent selection acting to remove the maladaptive effects of gene flow.

Pollen supplementation led to an increase in plant fitness rather than a decrease with increased soil temperature. Thus, the negative effect of soil temperature on fitness was largely mediated by pollinator availability and suggests that early flowering in heated soils might be maladaptive largely due to a mismatch with pollinators; i.e., plants in heated soils flower before most pollinators are active. Such a mismatch could possibly arise because *P. vulgaris* is more sensitive than its insect pollinators to soil temperature (Forrest and Thomson, 2011; Kharouba and Vellend, 2015; Pyke et al., 2016; Thackeray et al., 2016). Another possible explanation for a mismatch is that sessile plants in the warmest soils experience a larger degree of warming than mobile insects because after overwintering they sample the environment over a smaller area than their flying pollinators. Although the results of the pollen-supplementation experiment strongly suggest that the observed negative fitness effects of soil temperature were mediated by plant flowering time and plant–pollinator interactions, we cannot completely exclude the possibility that soil temperature also had direct, physiological, effects on plant fitness. Such direct effects of temperature on fitness could, for example, be investigated in experiments where plants are grown under controlled conditions where only soil temperature varies.

In contrast to the findings for pollen availability, the effects of soil temperature on fitness were not related to prey availability: fitness decreased with soil temperature whether the plant was supplemented with prey or not. This lack of an effect of prey availability might be because insect prey were available very early during the season in heated soils (i.e., plants and insect prey responded similarly to spring soil temperature) or because prey availability did not strongly limit seed production. The latter explanation appears to be in conflict with the previous finding that insect prey contributes a substantial amount of the annual nitrogen requirements of *P. vulgaris* (Karlsson et al., 1994). In the study species, leaves and flowers are differentiated a year in advance of their development, and previous studies have found that prey supplementation in one year increases size and seed production in the following years (Thorén and Karlsson, 1998; Worley and Harder, 1999). It is thus possible that any effects of flowering phenology on prey availability and effects of prey supplementation might become detectable only in subsequent seasons. However, in our study, prey supplementation did not influence the incidence of flowering and fruiting or rosette area in the following year (2021, authors' unpublished data). Taken together, our findings thus indicate that lower fitness and maladaptation in heated soils are partly driven by interactions with pollinators, but not with prey.

In agreement with our hypothesis, a significant effect of soil temperature on selection on flowering time was shown by the aster models. Earlier flowering was favored in colder soils and later flowering in warmer soils. A possible reason for this pattern is that in heated soils, phenological responses to soil temperatures result in plants starting reproductive development when air temperatures are still very low. Such an early start might be associated not only with a reduced pollinator availability, but also with a less-favorable abiotic environment, e.g., in terms of an increased exposure to freezing events that damage developing flowers (Pardee et al., 2018). As a consequence, a later flowering at a given soil temperature should be favored in soils that are on average warmer. Similar temperature-dependent selection on timing of reproduction was found in one of two study years for the perennial *Cerastium fontanum* in a similar

setting (Valdés et al., 2019; authors' unpublished data). It is thus possible that the selection for later flowering observed in heated soils in our study are not present every year, but might be absent in years when climatic conditions are more favorable and pollinator availability early in the season is higher. Anyway, the pattern observed in *P. vulgaris* suggests that selection over time acts to restore adaptive phenological responses on heated soils.

Even though the negative effects of soil temperature on fitness were largely mediated by pollen availability, earlier flowering was favored in colder soils and later flowering in warmer soils also in pollen- and prey-supplemented plants. Thus, the observed temperature-dependent selection was not only mediated by the investigated biotic interactions. Abiotic factors that might contribute to the observed selection include the relationships between soil and air temperatures and between soil temperatures and growing season length.

CONCLUSIONS

Our results are important for understanding how plant populations respond and adapt to changes in climatic conditions. We found that low pollen availability was a major reason why fitness decreased in warmer soils, while prey availability did not play an important role in a carnivorous perennial herb. The effect of pollen availability suggests that high thermal plasticity in flowering time might be maladaptive in heated areas because it results in mismatches with pollinators. Importantly, we found selection for modified phenological responses, in terms of later flowering, in heated but not in unheated areas. Taken together, the findings of this study contribute to our understanding of the conditions under which evolved responses might become maladaptive and of the agents that are responsible for such shifts. They also provide an insight into how selection acts to restore adaptive responses in novel environments. Such knowledge is indispensable for understanding and predicting long-term responses to warming and identifying the role of biotic interactions on the adaptation of plant populations to changes in climate.

AUTHOR CONTRIBUTIONS

J.E. and B.M. conceived the ideas and designed the methodology; V.F.H. collected the data; A.V. analyzed the data with input from J.E.; A.V. and J.E. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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