**Hengill project**

**Project theory:**

Warmer soil temperatures might lead to direct changes in selection on, for example on traits associated with: temperature optima, chilling requirements of established plants and dormancy breaking mechanisms in seeds.

Warming may also have direct effects on several plant traits, in terms of phenotypically plastic responses. Particularly important traits in this context are vegetative and flowering phenology. Specifically, we hypothesize that local soil warming leads to an earlier onset of vegetative growth and flowering.

Phenology, in turn, determines how plants interact with the abiotic environment (e.g. light, moisture etc.), as well as with the biotic environment, e.g. in terms of pollinators, herbivores and competitors.

Plastic responses of phenology to soil warming will thus change plant interactions with the abiotic and the biotic environments. As a consequence the selective environment will change and to the extent that there is genetic variation, we expect secondary evolutionary responses to warming. These changes might regard a number of traits.

First plastic responses to warming in terms of an earlier phenology will result in that plants during the spring will experience shorter days and lower air temperatures. This is expected to lead to: Changes in natural selection on the thermal reaction norms of vegetative and reproductive development. Specifically, we expect adaptations in response to this selection to be counter-gradient, i.e. genetic changes will be in a direction opposite to phenotypically plastic changes. We thus that plants adapted to warmer conditions will require higher temperatures to develop vegetative structures and to start flower (… elaborate why the optimal onset of vegetative and flowering phenology should change in response to warming in this way, analogously to responses to latitudinal differences … ?). We hypothesize that because growing season will be longer, warmer and more constant with regard to temperature at higher soil temperatures, there will be less strong selection for onset of growth and flowering at low temperatures than at lower soil temperatures. We thus predict that individuals from colder soils will grow more at low temperatures and respond more strongly to increasing temperatures than individuals from warmer soils in a controlled environment. [We also hypothesize that temperature will be less informative about future growth conditions at warm soils than at non-warmed soils. And predict that day length will be relatively more important as a cue than temperature in plants from warm sites compared with plants from non-warmed sites].

Second, assuming that pollinators and herbivores interacting with plants “sample” the environment over a larger spatial scale, soil temperature-driven changes in phenology also means that plants might experience changed interactions with pollinators and herbivores due to changes in synchrony. We that such changes in selection may result in response of several plant traits, including reproductive effort (including bulbils vs. flowers), flower number, flower size, flower morphology, self-compatibility system and plant defense. More specifically we hypothesize that plants from warmed soils will be less synchronous with pollinators and herbivores, and more often experience a lack of pollinators or damage by herbivores than plants from non-warmed soils. We thus predict that plants from warmer sites will be selected for increased selfing, e.g. having smaller flowers, being more self-compatible, and more able to self-fertilize, as well as being less defended.

**Project carry-out:**

In this project, we focus on a set of traits that we predict should be crucial for performance in a heated environment: vegetative phenology, flowering phenology, flower size, flower morphology, reproductive effort, self-compatibility system, herbivore defense mechanisms, chilling requirements of established plants, and dormancy-breaking mechanisms in seeds.

The project consists of four parts where we investigate:

1. Direct effects of soil warming on phenotypic selection of plant traits and responses to such direct selection in terms of genetic differentiation in chilling requirement for onset of vegetative growth in established plants, as well as for dormancy breaking in seeds.
2. Phenotypic plastic responses of plant phenology to warming.
3. Phenology-mediated changes in natural selection:
4. temperature-driven changes in selection thermal reaction norms of vegetative and reproductive development mediated by differences in phenology
5. temperature-driven changes in selection on reproductive effort (including bulbils vs. flowers), flower number, flower size, flower morphology, self-compatibility system and plant defense, mediated by interactions with pollinators and herbivores.[Specifically, we expect warmer soils to be associated with: (a) Altered (lowered) reproductive effort? (cf. allocation to flowers vs. bulbils in Bistorta) … (b) Altered (lower) investments in pollinator attraction: changed flower morphology – smaller flowers, more generalist-syndrome flowers; changed fragrance, … c) Also soil warming might lead to increased small-scale heterogeneity leading to increased benefits of a genetically diverse offspring, and thus of sexual reproduction, (d) Increased selfing, break-down of self-incompatibility systems … (e) Changes in defense compounds …]
6. Genetic differentiation in plant traits in response to such changes in selection.

**Design:**

**Study sites:**

In the proposed project, the overall aim is to examine the capacity of plants to respond evolutionary to environmental changes, such as warming. We will strive to overcome many of the limitations of previous studies by studying the effect on warming of plants in two geothermally heated ecosystems in Iceland. In these systems the soil is heated by the steam of deep geothermal water reservoirs, creating a relatively large gradient of soil warming (ambient to + 20°C over ambient) in an area of less than 500 m2 that does not differ in soil chemistry (see e.g. 6). As these systems have been heated for over 50 years (probably much longer) they offer a unique natural laboratories for studying the long term effects of warming on ecosystems.

The feasibility of using ecosystems that are heated by the steam of deep geothermal water reservoirs as a good proxy for climate change has already been demonstrated in previous studies both at the study sites (e.g. www. forhot.is6,15-17 ) and at other geothermal sites (e.g. 18). Soil warming will lengthen the growing season of plants e.g. by accelerating snow melt and soil thawing in the spring, which will influence nutrient cycling and microbial activity, the factors that are though to influence plant spring phenology (and other plant traits) the most in arctic ecosystems19. The PI has been working on the Hengill study site since 2014 and Grændalur since 2016. The PI is thus familiar with the ecosystems studied. Pilot studies have already been done on Hengill to check the feasibility of performing this study (more detail on them in section C). In addition, the PI and her collaborators possess the knowledge necessary for this project. Thus no obstacles for this project are apparent.

The study will be conducted at two sub-arctic geothermal sites in Ölfus municipality in SE-Iceland, Hengill (360 m.a.s.l) and Grændalur (100 m.a.s.l). Both sites offer a gradient of soil temperatures, from non-heated controls to over 20°C above ambient, over a short distance, without significant changes in other abiotic factors (e.g. soil chemistry, elevation). WP1 and WP3 will be performed at both study sites, WP2 only at Hengill.

**Study species:**

*Bistorta vivipara, Cerastium fontanum, Cardamine pratensis, Viola palustris, Thymus preacox, Pinguicula vulgaris, …*

**Field study:**

**Before the flowering season - Plots selection and marking of plants:**

* For each species, we want to mark 300 individuals. We want to sample individuals to cover variation at two different spatial scales: small-scale variation within heated areas, where variation probably is more dynamic, and in heated vs. non-heated areas, where differences are likely to be more persistent. Make sure there are a sufficient number of plots in heated and non-heated areas.
* Aim for 15 plots per species, 5 plots in non-warmed areas and 10 in warmed areas. At least 25 m between plots (if possible). The size of the plot is determined by the density of individuals so that each plot should include at least 20-30 individuals of the target species. If possible, the same plot could be used for more than one of the study species.

Make sure that variation within and among plots in warmed areas covers as broad range in soil temperature as possible. In the field, divide the temperature up to categories and have measured plants equally distributed among the categories. Try to have

Aim for at least 20 flowering individuals per plot. If individuals are marked at early bud stage it will be sufficient to mark just over 20 to compensate for losses before flowering. If individuals are marked before buds are visible (and thus we cannot be sure whether they will flower or not), then we need to mark more than 20 individuals – 30? - to have at least 20 flowering in the end.

* Mark plots with flags and metal rods.
  + Make sure to buy rods that do not rust (the nails we bought last year did rust a lot)
* Take the GPS-coordinates of each corner.
* Mark the position of each plot also on a map of the area.
* Within each of the 15 plots, mark 20 individuals. (This will result in 200 individuals in heated areas and 100 in non-heated.)
* Make a map of each plot, and mark the position of each marked individual on these maps.
* Measure the soil temperature at 5 cm depth in the immediate vicinity of each plant
* Ibuttons at some plants?
  + Put out x ibuttons per plot– The ibuttons will be placed next to x randomly chosen plants of each species in the plot, equally distributed among the temperature categories.
* Record plant individuals at regular intervals following a rolling scheme – the intervals will depend on time needed to complete one round of recordings. Once every week would be the target. See next section.

**During the flowering season in all marked individuals –**

**Record reproductive development stage at each visit:**

1. Reproductive stage – measure will differ between plant species (see below). Reproductive stage will be followed in terms of the stage of the most advanced bud/flower until the opening of the first flower, and in terms of the date of peak flowering of an individual, i.e. the date when at least 50% of all flowers of an individual have opened.

**Record other traits once:**

1. Vegetative size – measure only once, at the start of flowering; measure will differ between plant species (see below)
2. Flower number – only needs to be done once, and then at a time when flowers are relatively easy to count. However, in some species it might be hard to find a single occasion when this is feasible (see species-wise details below). For Bistorta, also count the number of bulbils at the same time as when flowers are counted.

**After the flowering season - at seed maturation:**

1. Count the number of mature intact fruits on the plant individual and note it in the protocol.
2. Collect up to 3 fruits from each individual plant, note the number of fruits collected in the protocol, and put them in separate envelopes. Take care to mark the identity of the individual and date of seed collection of the envelope

(3) Store the envelopes in a reasonably cold and dry place.

Collect leaf samples from Cardamine sometime during the growing season, the earlier the better. Note temperature for sampled individuals (see separate protocol).

**Species-wise details for recordings (see the google doc):**

*Bistorta vivipara*

1. Vegetative size – as Maya did, number of leaves and length and width of largest leaf? - check
2. Reproductive stage of the most advanced flower bud/flower at each recording until the opening of the first flower, AND the date of peak flowering of an individual, i.e. the date when at least 50% of all flowers of an individual have opened.
3. Flower and bulbil number – count or some rougher estimate?

*Cerastium fontanum*

1. Vegetative size – Rosette diameter, number of flowering stems and median height of flowering shoots (i.e. the height of a representative shoot) at start of flowering
2. Reproductive stage of the most advanced flower bud/flower at each recording until the opening of the first flower, AND the date of peak flowering of an individual, i.e. the date when at least 50% of all flowers of an individual have opened.
3. Flower number –– flower number at time x (then maybe also take this in the end of the season)

*Viola palustris,*

1. Vegetative size – number of leaves and length and width of largest leaf?
2. Reproductive stage of the most advanced flower bud/flower at each recording until the opening of the first flower, AND the date of peak flowering of an individual, i.e. the date when at least 50% of all flowers of an individual have opened.
3. Flower number – counting all flowers

*Pinguicula vulgaris*

1. Vegetative size – Rosette size AND height of inflorescence at start of flowering
2. Reproductive stage of the most advanced flower – bud size classified in different categories (see separate protocol for this), open, wilting
3. Flower number – counting all flowers

*Thymus preacox (Copy and adjust from Nia)*

1. Vegetative size – Thymus is a cushion plant so we need to adjust the sampling method to that (Nia used some good protocols last summer and they were based on the literature we can just follow them).
2. Reproductive stage of the most advanced flower bud/flower at each recording until the opening of the first flower, AND the date of peak flowering of an individual, i.e. the date when at least 50% of all flowers of an individual have opened.
3. Flower number – might be difficult to count at a single visit, rough estimate? (Nia had some good protocols)

*Cardamine pratensis*

1. Vegetative size – Rosette diameter, number of flowering stems and median height flowering stems at start of flowering
2. Reproductive stage of the most advanced flower bud/flower at each recording until the opening of the first flower, AND the date of peak flowering of an individual, i.e. the date when at least 50% of all flowers of an individual have opened.
3. Flower number – counting all flowers

**Material needed for field work:**

*Before:*

GPS

Maps of the area

Markings for plots (4\*30 depending on how many plots)

* Metal rods (Beygt steypustyrktarjárn)
* Wooden poles (Where to get)

Marking for individuals

* Nails make sure to buy rust free ones (1800 nails)
* Making of plastic tags (takes time)

3-4 Soil thermometers

Ibuttons!? let us say we have 30 plots and want 4 ibuttons per plot. That is 120 ibuttons. The cheapest ones come at about 10-12 Euro I think, That would mean about 1200 – 1500 Euro. I think that is possible for me to pay. However, loggers that can record for a year without charging is a bit more expensive I think.

*During:*

Rulers for measurements of plant size

Rulers for measurements of flower size

Magnifying glasses

Protocols

Car (probably from 15 May to 15 July – get an offer – BM has sent an email about this)

*After:*

4500 bags for seed collections – we need to order bags, do you have a good supplier (I have not found any in Iceland jet, but we should be able to order from Amazon, but then it is allot of taxes etc. maybe better if you can buy in Sweden and carry here in the luggage).

**Time plan**

X = Mark plants

Black = Trait measurements

Gray = Phenology measurements (once a week) – light gray: few/no plants with phenology measures (not flowering or wilted) – dark: most plants with phenology measurements

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | May | | | | June | | | | July | | | | August | |
|  | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |  |  |
| Viola |  | x |  |  |  |  |  |  |  |  |  |  |  |  |
| Cerastium |  |  | x |  |  |  |  |  |  |  |  |  |  |  |
| Cardamine |  |  | x |  |  |  |  |  |  |  |  |  |  |  |
| Pinguiculia |  |  |  |  | x |  |  |  |  |  |  |  |  |  |
| Bistorta |  |  |  |  | x |  |  |  |  |  |  |  |  |  |
| Thymus |  |  |  |  | x |  |  |  |  |  |  |  |  |  |
| FA1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| FA2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| FA3 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

* **In lab:**
* Record fitness in all individuals – ideally seed number but possibly number of intact developed fruits per marked individual
* This data can be used to estimate phenotypic selection on all recorded traits, and to assess how phenotypic selection varies with soil temperature
* It is also possible to estimate the fraction of selection mediated by pollinators and herbivores by manipulating the level of pollen limitation and the level of herbivory experimentally in separate experiments

**Cultivation parts:**

* Grow progeny in green-house and common garden
* Assess the amount of genetic variation present in populations for all relevant traits – common garden experiments, and greenhouse experiments for self-incompatibility and defense compounds. Traits to examine:
* Temperature optima
* Chilling requirements of established plants
* Chilling requirements of seeds
* Thermal reaction norm of vegetative growth
* Thermal reaction norm of flowering time
* Reproductive effort (veg vs. sex)
* Flower size
* Flower morphology
* Self-incompatibility
* Fragrance
* Defense compounds
* Genetic differences in fixed traits among individuals from different soil temperatures will be assessed in common garden experiments
* Genetic differences in thermal reaction norms will be assessed in series of climate chamber experiments
* Genetic differences in chilling requirements will be assessed in experiments in greenhouse/common garden/climate chambers/fridges/freezers

**Transplantation experiments!?**