

Leveraging newly available data to quantify gene x environmental control on plant phenology

1. INTRODUCTION

Shifts in plant phenology (the timing of flowering, leaf-out, and other life history events) have been extensively documented as occurring in response to climate change and can include observational data that are several centuries old¹. Shifts in spring plant phenology are extremely varied in magnitude and direction but overwhelmingly favor shifts to earlier events associated with warmer spring temperatures². Furthermore, these shifts are strongly associated with changes in individual plant performance³⁻⁷, altered species interactions^{3,8}, and changed population-level demographic processes^{9,10}.

Despite the widespread study of phenological shifts and the consequences for broader plant ecology, relatively little is known about the exact mechanisms underlying inter- and intraspecific variation in phenological sensitivity. Recent evidence suggests, and the literature is in relative consensus, that plant phenology is the result of some combination of (1) genetic constraints and (2) phenotypic plasticity, but the ratio of contributions from these two processes remain largely untested outside of a few species¹¹. This is problematic because differences in their relative contributions are expected to have vastly different consequences on species' abilities to track climate change via range shifts^{11,12}. Thus, improving our understanding of the tradeoffs between plasticity and local adaptation will greatly facilitate our ability to conserve plant species, especially those with narrow or threatened ranges.

Here, I propose to make use of existing long-term datasets collected in Asia (Korean and Japan weather station phenology data) and Europe (PEP725 database) to identify a signal of plastic effects of plant phenological sensitivity across a group of 33 species, and to disentangle these effects from those of local adaptation. These historical datasets are unique in their control for genetic variation and have only become publicly available within the past decade. Because of my extensive background working with historical, intercontinental phenological datasets and my extensive international network of colleagues, I am uniquely positioned to conduct the intercontinental analysis proposed below.

2. SPECIFIC AIMS

This project will provide new insight into how plasticity and local adaptation interact to influence plant phenological sensitivity to global climate change. I will use genetically controlled observational data in combination with genetically variable herbarium and crowd-sourced observational data to test three aspects of the mechanistic underpinnings of phenological shifts in temperate deciduous plant species:

- 1) Baseline: Quantify baseline phenological sensitivity using genetically controlled, decades-long observational datasets in Europe and Asia.
- 2) Mechanism: How “perfectly plastic” phenological sensitivity differs from that of wild plants subject to natural adaptation to local environmental conditions (hereafter “genetically variable”)
- 3) Forecast: Implications of how climate change will affect plant phenology given new knowledge of interactions between plasticity and adaptation.

My aims and approach build on previous research and collaborations, particularly relating to phenology and climate change with my UK sponsor Dr. Will Pearse¹³.

3. RATIONALE AND METHODS

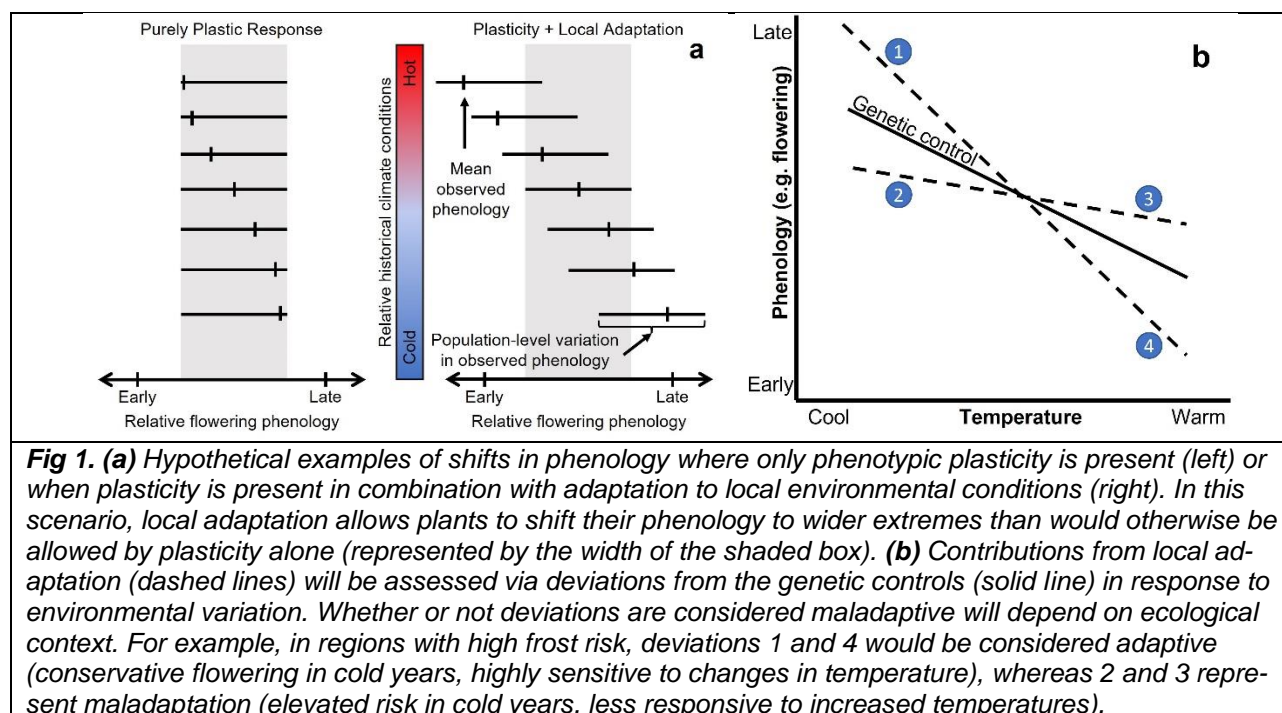
(1) Baseline: The crux of the proposed project is a comparison of the phenological sensitivity in groups of plants that differ in whether they are genetically controlled (i.e., whether all plants within a study group are genetically identical or otherwise are composed of individuals that are subject to natural patterns of adaptation to local environmental conditions). Genetic controls in long-term phenology observational datasets are rare, but we know of at least three that either account for or control for genetic diversity in datasets spanning between 60-100 years. The first two of these datasets are each located in east Asia (Korea and Japan) and include observational data collected at phenological gardens associated with weather stations (see refs^{13,14} for previous use of these data in ecological studies) for a total of 8 woody plant species. For both, individual plants at weather stations were planted from common stock (which is maintained to this day and used to replace damaged/dead individuals), meaning that they are genetically identical across the extent of the observational network and throughout the extent of the study. Data collection began in Korea in 1920 and in Japan in 1953.

The third dataset is from Europe and is currently part of the PEP725 phenological database (<http://www.pep725.eu/>). As recently documented by ref¹⁵, phenological data have been recorded for 23 woody plant species in phenology common gardens across Europe since 1959. Similar to the Asian datasets, initial plantings of 27 species were clones and so were genetically identical across the study range and throughout the temporal extent of the observations. However, this European network differs in that they planted multiple genotypes of many of the species, with all but four of the species having between 2-8 genotypes present. These data were also collected over a broader geographic range compared to the Asian data, with phenological gardens ranging from Ireland to Estonia and from Portugal to Norway¹⁵.

Using previously validated analytical approaches^{10,13,16,17}, I will quantify species-level phenological sensitivity in each dataset (i.e., the change in phenological timing in response to environmental change. I will focus

solely on day of first flowering (FFD) in spring, as it is a common variable across all three datasets, and investigate the contribution of various drivers that have been shown to be important cues of spring phenology (e.g., temperature forcing, winter chilling, and photoperiod). **I will therefore produce a genetically controlled, intercontinental, and temporally extensive ecological baseline representing the “pure plastic” response of plants to environmental variability over the past ~60-100 years.**

(2) Mechanism: The second goal of this research is to identify how the effects of phenological plasticity identified in part (1) differ from and interact with the effects of local adaptation. Plants often show signals of genetic adaptation to long-term, historical climate trends¹⁸. For example, a recent study found that trees and herbaceous understory plants in eastern North America had different phenological sensitivity to spring warming in different parts of their ranges¹⁹. Furthermore, the degree of phenological plasticity that plants exhibit has also been shown to be affected by patterns of local adaptation²⁰, suggesting that plasticity, itself, is also an adaptive trait. Because ecological processes such as species distributions and range shifts can be shaped differently by the relative contribution of these two processes¹¹, it is important to disentangle the signals of plasticity and genetic differentiation in patterns of phenological sensitivity (Fig 1a).



This will not be the first study to address this knowledge gap, but it will be the most comprehensive to date and the first, to my knowledge, to utilize historical datasets in this pursuit. Past studies have been limited in phylogenetic and spatiotemporal extents (no more than three species per study and typically constrained to local or regional levels over the past decade). **Only now, with newly digitized and publicly available historical datasets, can we conduct evaluations of how plasticity and genetic differentiation interact to affect plant phenology within a long-term global context.** Here, I propose to disentangle the effects of plasticity and genetic differentiation by contrasting phenological sensitivity in natural populations to cloned plants in phenological gardens described above.

Specifically, using the same cohort of European and Asian plant species present in the part (1) datasets, I will aggregate phenological data from herbarium collections that have been amassed over the same or longer time periods and in the same regions where the observational phenology data were collected. Herbarium collections are commonly used in studies of plant phenology¹⁶ and have been extensively validated against observational phenology data²¹. Their use will allow for insight into the variation in phenology in genetically variable individual plants, thereby providing information about phenological sensitivity when affected by both plasticity and genetic differentiation. I will compare the trends from part (1) to the trends in herbarium-derived phenological sensitivity to infer the role of local adaptation in determining phenological sensitivity (Fig 1b).

Importantly, there are multiple possibilities for the deviations between trends (e.g., deviations from the genetically-controlled lineages could indicate either positive or counter-gradient adaptation^{22,23}). These distinctions will be easier to make in the European dataset, which contains multiple controlled lineages in the observational dataset, but will be more challenging to make in the Asian datasets, where only a single lineage per

species per country is available. Still, overlap in species included in the Japanese and Korean datasets, as well as detailed information about species sources, should be enough to be able to make these distinctions.

(3) Forecast: After disentangling the roles of plasticity and genetic differentiation in determining phenological sensitivity, I will then use information from my models to extrapolate how these differences will continue to affect plant phenology in future decades and discuss the ecological implications for these differences. I will use climate change forecasts provided by the Intergovernmental Panel on Climate Change, aggregated by WorldClim (worldclim.org), an approach that I have used successfully in previous publications^{10,16,24}. I will use several different climate change scenarios to provide a wide range of predictions that span possible foreseeable climate change between now and the end of the 21st century.

4. PRELIMINARY DATA/ANALYSIS

As part of my current collaboration with my sponsor (Dr. Will Pearse), we already know that the “purely plastic” phenological sensitivity (i.e., the sensitivity to temperature of genetically controlled plants) is

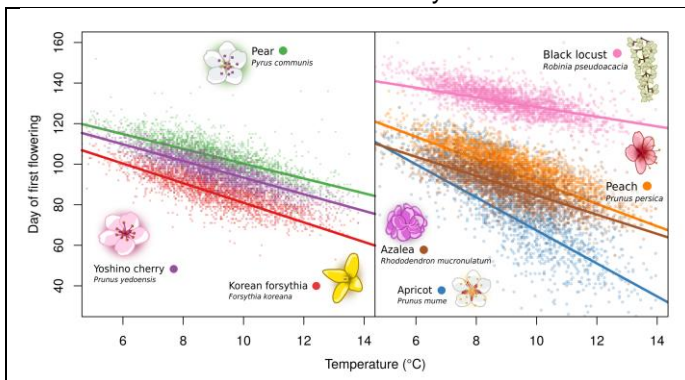


Fig 2. Previous phenological trends from the genetically controlled Korean weather station phenology dataset¹³. Trends were found to be more linear than expected, and consistent across all 100 years of the observational period.

very linear and more linear than expected in natural populations¹³, see Fig 2), at least in Korea. Assuming we find similar trends in the Japanese and PEP725 data, we can then identify deviations from these genetically controlled trends in the naturally varying datasets (herbarium records and crowd-sourced phenology; both of which I use extensively in past and ongoing research projects)^{16,24}.

5. OUTREACH AND PATHWAYS TO IMPACT

I will work closely with my sponsor to engage in educational outreach activities in the United Kingdom that make use of my extensive experience working as an educator in the U.S. Specifically, I will participate in the Great Exhibition Road Festival (<https://www.greatexhibitionroadfestival.co.uk/>) and Bugs, Birds, and Beasts Day (<https://www.imperial.ac.uk/georgina-mace-living-planet/events/bugsdays/>). I will also receive media and public policy training from the Grantham Institute to build on my skills as a science communicator. Lastly, results of my study are likely to have important implications for forestry and agriculture, and so I will leverage my sponsor’s connections to Syngenta and ADAS to ensure my findings are communicated to agencies leading public policy development and forestry management. Phenology plays an incredibly important role in sustainable food production and I will work with these industries to further secure the UK’s food future.

6. TIMELINE (Proposed start date: Feb 1, 2024)

	2024				2025				2026			
	1	2	3	4	1	2	3	4	1	2	3	4
Move to UK, initiate research	█											
Acquire updated historical datasets, clean the data		█	█	█								
Establish genetically controlled baseline sensitivity												
Visit Korean herbarium, score specimens (3 wks)												
Visit Japanese herbarium (3 wks)												
Aggregate/score herbarium data (EU + Asia)												
Herbarium phenological sensitivity statistical analysis												
Writing and publication												
Conference presentations (BES, ESA)												
Great Exhibition Road Festival (June)												
Bugs, Birds, and Beasts Day (July)												
Media and public policy training												

Refs: (1) Aono (2015) *Int J Biometeorol*; (2) Piao et al (2019) *Global Change Biol*; (3) Heberling et al (2019a) *New Phyt*; (4) Kwit et al (2010) *Can J For Res*; (5) Lee & Ibáñez (2021a) *Funct Ecol*; (6) Münzbergová et al (2017) *J Ecol*; (7) Seiwa (1998) *J Ecol*; (8) Kharouba & Vellend (2015) *J Anim Ecol*; (9) Ibáñez et al (2017) *Oecologia*; (10) Lee & Ibáñez (2021b) *Global Change Biol*; (11) Zettlemyer & Peterson (2021) *Front Ecol Evol*; (12) Ghalambor et al (2007) *Funct Ecol*; (13) Pearse et al (in review); (14) Ibáñez et al (2010) *PTRS B: Biol Sci*; (15) Renner & Chmielewski (2022) *Int J Biomet*; (16) Lee et al (2022) *Nature Comms*; (17) Pearse et al (2017) *Nature Eco Evo*; (18) Zohner et al (2017) *Ecol Letters*; (19) Miller et al (2022) *J Ecol*; (20) Ensing & Eckert (2019) *New Phyt*; (21) Ramirez-Parada et al (2022) *Ecography*; (22) Eckhart et al (2004) *Evolution*; (23) Conover et al (2009) *Annals New York Acad Sci*; (24) Yang et al (2022) *Int J Plant Sci*