Introduction:

Herbarium collections are increasingly becoming important to long-term ecological studies, as they can provide a wealth of information about plant traits and species ranges (Heberling, 2022), and can help us understand the historical context and consequences of climate change. Recently, there has been particular interest in using these collections to relate plant phenology (i.e., the timing of repeated life history events such as flowering, leafing, and fruiting) to environmental variation. Such studies can provide critical information about shifts in phenology in response to climate change (Lee et al., 2022) and spatial distributions (Willems et al., 2022), and they can provide important insight into species interactions (Lee et al., 2022; Miller et al., 2022). In short, herbarium studies offer unique leverage into how plants function and interact over long time spans and wide geospatial gradients.

An important recent application of herbarium-based ecology has been to explore how flowering period overlap in dioecious species changes over time and space (Yang et al., 2022). Dioecious species are those that have male and female flowers on separate plants, and successful reproduction in these species is thus highly dependent on male flowers, female flowers, and associated pollinators all occurring at the same time and location. Mismatch in phenology of any of these three groups would lead to unsuccessful pollination, reduced overall fitness, and declines in demographic performance.

Importantly, dioecious species may be particularly threatened by climate change because male and female plants tend to have different responses to environmental variation. For example, male/female photosynthetic ratios and stomatal conductance ratios differed between control and climate change treatments (drought and/or warming treatments; Hultine et al., 2016), and were speculatively linked to observed population-level sex ratios. Moreover, I (and colleagues) recently theorized that disproportionate phenological sensitivity between male and female plants could also lead to disruptions in long-term population stability (Yang et al., 2022). Dioecious plants are frequently offset in the start of their flowering period by days to weeks, albeit with substantial overlap in male and female flowering periods (e.g., Dupont & Kato, 1999). If male and female plants are differently sensitive to environmental conditions that cue their flowering phenology, the length of this overlap period could shorten and lead to reduced pollination rates and reproductive success.

In our recent paper, colleagues and I used herbarium specimens of dioecious blunt-lobed spicebush (*Lindera obtusiloba* Blume) to investigate the potential for flowering mismatch in Chinese herbarium specimens. Interestingly, we did not find a signal for different phenological sensitivity between male and female plants, but we did find evidence for a bias in collection dates between the sexes, where male plants were collected on average 25 days earlier than female plants (Yang et al., 2022). This was unlikely to be reflective of natural processes as the sexes are typically only separated in timing of peak flowering by about 10 days, suggesting a collection bias. Additionally, we were limited to a sample size of only 88 specimens, making it difficult to separate the climate change signal from noise associated with geographic and temporal variation.

Goals: Here, I propose to work with **Dr. Laura Russo at the University of Tennessee – Knoxville** to broaden this study of dioecious species' phenological sensitivity to environmental conditions using species native to and common throughout eastern North America. This will entail visiting the UTK herbarium to sex the dioecious plant collections, which is difficult to do from digitized images alone. I will also extend this analysis using collections housed at Carnegie

Museum of Natural History, where I currently work. This will increase our explanatory power and reduce sampling bias present for our analysis of the Asian *Lindera* species. I also plan to extend this analysis to three dioecious plant species. Lastly, I will examine the phenological sensitivity of pollinator species (*Andrena* spp.) that specialize on one of the proposed plant genera (*Salix* spp.) using specimens collected by insectaria in the greater Appalachian region.

Methods: I will score phenological data and sex flowers for three different dioecious species that are common through eastern North America: *Lindera benzoin* L., *Aralia nudicaulis* L., and *Salix nigra* Marsh. All three species are relatively common, with a total of 633, 427, and 536 digitized specimens, respectively, in the UTK and CMNH herbaria. Distinguishing between male and female flowers is difficult to do with digitized images and will therefore require me to travel to Knoxville at least once to sex the plants in their collection. Analysis of sex-specific phenological sensitivity will follow previously established methods (Lee et al., 2022; Yang et al., 2022), largely relying on freely accessible historic climate estimates.

One of the species, *Salix nigra*, is of particular interest to this research because willows serve as an important early-spring pollen resource for native bee populations (Mitchell et al., 2022; Ostaff et al., 2015). Of particular interest to my collaborator at UTK (Dr. Laura Russo) are sand burrowing bees in the genus *Andrena*, which are oligolect pollinators of *Salix* (i.e., they consume exclusively willow pollen; Tumminello et al., 2018). Therefore, I will supplement my analysis of plant phenological sensitivity with an analysis of *Andrena* phenological sensitivity using historical collections housed at CMNH and at the Frost Entomological Museum at Pennsylvania State University. This approach of comparing pollinator and dioecious plant phenological shifts within the same study is, to our knowledge, completely novel, and will provide us with additional evidence with which to assess potential reductions in reproductive success associated with plant-pollinator mismatch.

Significance: Dioecious plant species compose a polyphyletic group with species found in over half of all plant families (Renner, 2014). Accounting for about 6% of all angiosperm species studied, they are an important component of plant biodiversity and an important pollinator resource in natural and managed systems. And while many studies compare dioecious flowering phenology between male and female plants of the same species (Dupont & Kato, 1999), there is a general lack of knowledge about if and how flowering responds to environmental variation across time and space (Yang et al., 2022). The proposed study will offer novel and important insight into phenological sensitivity in dioecious North American plants of different growth forms (an herbaceous wildflower, a shrub, and a tree). Furthermore, this study will have the additional novelty of comparing not only male and female plant sensitivity, but oligolect pollinator phenology as well, which has not yet been done to the best of my knowledge. Generating this information is important for furthering ecological theory and for furthering our understanding about how temperate forests will respond to climate change.

Importance of UTK herbarium: The UTK herbarium is an important plant collection with over 649,000 specimens and is situated at the latitudinal center of eastern North America, the range over which this study will be conducted. Dioecious plants are often difficult to sex from digitized images alone, so visiting this herbarium (and conducting an identical analysis at my home institution) will be necessary to answer the questions posed for this project.

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Itemized budget:

- $500 for travel between Knoxville, TN and Pittsburgh, PA
- $1,000 for lodging in Knoxville
- $500 for *per diem* expense during herbarium data collection in Knoxville
- $500 for travel, lodging and *per diem* expenses associated with trip to Frost Entomological Museum/Insectarium
- $500 for publication costs and/or conference travel expenses related to publishing and presenting results from resulting analysis.

Total Budget: $3,000