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Systematic Map Protocol

Title
What pollinators serve federally-listed imperiled plants in the Southeastern US?

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Background
The Southeastern United States is a known hotspot for plant diversity, and the region's plants have become threatened due to habitat loss, degradation, and fragmentation (Ross et al. 2014, Belote et al. 2017). This leaves many flowering plants in habitats classically associated with poor pollination outcomes (Wilcock and Neiland 2002), which can diminish population growth and hinder conservation efforts for imperiled species. Habitat loss and degradation frequently leave plants in small, isolated populations unable to attract pollinators (Lamont et al. 1993, Groom 1998), and fragmentation can shift pollinator communities and their interactions with flowers (Cunningham 2000). Alternatively, many imperiled plants experience loss due to disturbances, like fire, which can affect pollinator availability (Carbone et al. 2019). However, flowering plants often do not depend on specific pollinators (Johnson and Steiner 2000, Brosi 2016), and flowers may find effective pollinators despite habitat changes, confounding the need for recovery actions to include pollinators. Because of these concerns, pollination studies are undertaken for many imperiled plants in the Southeastern US, especially those federally-listed as endangered or threatened, and this has produced a body of literature ripe for mapping to identify common pollinators for imperiled plants in the Southeastern US. We plan to map this evidence base, and we anticipate recovery biologists and botanical gardens can use this map to integrate individual species into region-wide plans to protect plants and pollinators. This map will also address general concerns about imperiled plants, specifically whether they rely on certain pollinators that may be declining across the Southeastern US.

Theory of change or causal model
While many flowering plants can reproduce without pollinators, several species are dependent on animal pollinators, particularly woody perennials and those with multiple reproductive episodes, and these plants are vulnerable to lower fertility in the absence of pollinators (Rodger et al. 2021). Many federally-listed imperiled plants share these qualities, suggesting that recovery actions should include pollinators, but it is unclear if and how to include them because there is no synthesis of pollinators for imperiled species. This map seeks to identify what pollinators serve imperiled plants, and this data can be interrogated further to ask questions about pollinator shifts and losses.

Stakeholder engagement

Three map authors (BC, KGV, and RG) conceived of the map question after several informal consultations with state and federal recovery biologists. Recovery teams conveyed data gaps about biotic interactions, particularly pollination, and ambiguity about how pollination should be addressed in recovery plans. Two authors (ESK and PMJ) are federal recovery biologists charged with identifying pollinators of importance to listed species for the Southeastern US, an effort aligned with broad federal interests to protect pollinators. They shared specific concerns about study design and plant biology that directly informed critical appraisal of studies and secondary questions that will be used to identify patterns from the map database. In addition, ESK and PMJ work within USFWS's National Wildlife Refuge system and want to understand how to protect pollinators for imperiled species within the Refuge network.

Objectives and review question

This map aims to identify pollinators common to plants listed as endangered or threatened in the Southeastern US. Primary question: What pollinators are known for federally-listed endangered or threatened plants found in the Southeastern US including Puerto Rico and the US Virgin Islands? Secondary questions: What methods were used to determine pollinators? What stage of pollen movement do these methods observe? Where were studies conducted? How many populations and individuals were included in each study?

Definitions of the question components

Population: any federally-listed endangered or threatened flowering plant found within US Fish and Wildlife Service (USFWS) Region 4 (Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, Puerto Rico, South Carolina, Tennessee, and U.S. Virgin Islands), Exposure: any animal visiting flowers within plant population of interest, Outcome: seeds or fruits produced after pollen movement

Search strategy

We anticipate relevant studies to be found among peer-reviewed articles, technical reports, dissertations, and theses. We plan to search research databases, snowball-search reference lists of eligible studies, and hand-search Google Scholar results, regional science journals, USFWS five-year reviews, organizational websites, and an internal USFWS database. We will use a P-E-O question structure where population is plant, exposure is animal, and outcome is seed or fruit produced. Flowers have many animals that visit them, but not all visits result in seed or fruit production. This means that visitor and pollinator communities are distinct groups of animals, and we specifically seek studies that differentiate between visitor and pollinator. Population terms are scientific names for federally-listed imperiled plants and their synonyms as given by Integrated Taxonomic Information System (ITIS) and Global Biodiversity Information Facility (GBIF). We will include all imperiled flowering plants within USFWS Region 4 as of June 2023. Exposure terms are generic names and synonyms for floral visitors (e.g. bee, syrphid) and insect, bird, and bat orders known to visit flowers. Outcome terms include quantitative descriptions of pollen movement by animals. Appendix A includes our search queries. We use all default options for all research databases, which includes title, abstract, keywords, and full-text possible for a given database.

Bibliographic databases

We will query research databases for relevant peer-reviewed articles. Auburn University (PH) will search BIOSIS, Web of Science Core Collection, Zoological Record, and CAB Abstracts. Auburn's institutional subscription to BIOSIS covers from 1926 to present, Web of Science Core Collection from 1900 to present, Zoological Record from 1864 to present, and CAB Abstracts from 1910 to present. Mississippi State University (BB) will search SCOPUS, Academic Search Premier, Agricola, and Environment Complete, which only provide a single option of time periods covered. We will not apply any limiters to database searches, and we will not use the "Apply equivalent subjects" option in EBSCO. We will query for dissertations and theses in Proquest Dissertations & Theses Global, Digital Commons, WorldCat, OlAster, and D-Space repositories. When the protocol was first submitted to PROCEED, the authors also indicated that Wildlife & Ecology Studies Worldwide and Academic Search Complete would be searched. However, the MSU Libraries has decided to discontinue, effective June 30, 2023, their subscription to those two databases, though it will provide access to Academic Search Premier. Accordingly, BB will not search Wildlife & Ecology Studies Worldwide and Academic Search Complete for the study, but he will search Academic Search Premier. In the pilot searches, these databases provided no unique records.

Web-based search engines
We will search for conventional scientific and gray literature in Google Scholar. We will individually query each species name and its synonyms in Google Scholar as this search engine does not perform well using Boolean logic. We will search the first ten pages of search results for relevant literature.

Organisational websites
We will search five-year reviews archived in USFWS's Environmental Conservation Online System (ECOS) for conventional scientific and gray literature relevant to our map question. We will access web pages for each plant species through this website, and we will hand-search reference lists from the most recent five-year reviews and other listing documents (e.g., petitions, recovery documents) for relevant literature. We will also hand-search by plant species in two web-based databases for plant-animal interactions: Global Biotic Interactions or GLOBI (www.globalbioticinteractions.org) and Center for Plant Conservation's Plant-Pollinator Explorer (https://plant-pollinator.shinyapps.io/shinyapp/). Within the USFWS Region 4 Inventory and Monitoring Branch of the National Wildlife Refuge, ESK and PMJ have created an internal database of listed plants and their pollinators, and we will hand-search this database by plant species for relevant studies. ESK and PMJ will provide an open-source copy of this database that excludes sensitive agency information as a part of our map output. All websites are available in English.

Comprehensiveness of the search
To determine the comprehensiveness of the search strings, BC developed a test list of 16 relevant publications for 21 out of 153 federally-listed imperiled plants in FWS Region 4. The test list includes representative literature we want to include in our systematic map, and some studies include multiple imperiled plants, leaving fewer studies than plant species within our test list. We completed our pilot study on a subset of FWS Region 4 plants because including all plants would have complicated how we assess outcomes of search strategies. PH and BB developed a search string with population terms based on taxonomic orders and common names of floral visiting animals, exposure terms based on scientific names of plants including synonyms, and outcome terms based on common descriptions of pollination services by animals. For birds and bats, we searched by taxonomic family as birds and bats each have only one family that are likely pollinators in North America (Cronk and Ojeda 2008, Fleming et al. 2009). The following databases were searched: BIOSIS, CAB Abstracts, Web of Science Core Collection, Zoological Record, Scopus, Academic Search Complete, Agricola, Environment Complete, Wildlife & Ecology Studies Worldwide, ProQuest Dissertations & Theses, and WorldCat. BB checked the following components in WorldCat during the search: OAlster, PapersFirst, ProceedingsFirst, WorldCat, and WorldCat Dissertations & Theses. All items on the test list were found in bibliographic databases. Our benchmark list and pilot results are found in Appendix B.

Search update
We will update our search if more than one year passes between publication of this protocol and our final report.

Screening strategy
Two reviewers (BC and KGV) will screen literature from each search strategy independently using our eligibility criteria. Prior to screening literature from research databases, we will combine retrieved literature into a single Zotero folder and then de-duplicate results in Zotero. We will export de-duplicated results to Covidence and complete a check for reviewer consistency prior to screening. Screening will occur in two stages: first by title and abstract and then by full-text. During each stage, we will keep Covidence in blind mode so individual reviewer decisions remain hidden from the other reviewer, and we will assign reasons to exclude studies in Covidence. After we complete each stage, we will remove blind mode in Covidence to discuss conflict literature. When we complete screening studies from databases, we will search reference lists of eligible studies (i.e. snowball search) and manually enter retrieved studies into Zotero. Since we will initially select studies based on their titles, we will not formally screen titles and abstracts as conducted earlier but rather screen only full-texts in Covidence. After snowball searching, we will hand-search for relevant literature in Google Scholar, regional science journals, organizational websites, and an internal USFWS database. We will only screen full-texts from hand-searching as we would have initially selected studies based on titles. Interrater variability will not be tested after snowball and hand-searches because we would have ensured consistent agreement earlier while screening studies from databases.

Eligibility criteria
We will screen titles and abstracts based on population and exposure elements. We will screen full-texts using outcome elements and study language. Eligible titles and abstracts are likely to represent a mix of studies about floral visitors and pollinators as outcome elements are usually not reported in abstracts but rather in methods and results sections. We have noticed that some abstracts are indexed in English, but their full-text is in a different language. Consequently, we will screen full-texts by study language, which must be English or Spanish. We will not use study structure in our selection criteria because eligible studies will range in their approaches to differentiate floral visitors from pollinators.

Consistency checking
To assess agreement between screeners, we will complete a Kappa analysis after screening 10% of titles and abstracts obtained from research databases (James et al. 2016). If the Kappa score is greater than 0.6, we will screen remaining titles and abstracts. If the Kappa score is under 0.6, we will discuss conflict literature to re-evaluate how each screener interprets selection criteria, and we will complete a second Kappa analysis on 20% of remaining titles and abstracts. We anticipate this second Kappa analysis would yield a score greater than 0.6 and we can complete this stage of screening. We will repeat this check prior to screening full-texts of studies from research databases. We will report Kappa statistics from each screening stage in our map output.

Reporting screening outcomes
We will report eligible and ineligible studies in our map database, and for ineligible studies, we will include at which stage they were excluded and why they were excluded. If studies were found eligible after screening for titles and abstracts but we could not obtain its full-text, we will exclude these studies from full-text screening, and we will compile titles and abstracts from these studies in a separate list as a part of our map database (James et al. 2016). We will summarize the number of studies included and excluded after each screening stage following ROSES standards (Haddaway et al. 2018) and present this data as a PRISMA diagram in our final report (O'Dea et al. 2021).

Study validity assessment
Traditionally, critical appraisal of studies is not needed to map evidence (James et al. 2016), however, pollinator studies vary widely in how they identify pollinators, affecting validity of

inferences drawn from these studies. Pollen movement is hard to observe directly, and many studies rely solely on observational surveys of floral visitors. But visitation does not directly relate to pollen movement even when animals are frequent visitors (King et al. 2013). Studies that identify pollinators by observation are less reliable than studies that employ other methods that measure pollen movement by animal visitors. Consequently, including these studies would reduce confidence in ecological patterns drawn from map synthesis. Prior to data extraction, we (BC and KGV) will classify how studies identified pollinators, specifically asking whether a study only used observation to identify pollinators. We will differentiate observation-only studies from studies that measured pollen movement, and these studies will not be included in data extraction or synthesis. We will report how many observation-only studies were excluded and list their bibliometric data in our map database. We will not appraise if studies confirmed their study species benefits from animal pollination. This systematic map does not seek to identify patterns of plant mating among imperiled plants as addressing this requires a different mapping approach. Rather we assume that study authors confirmed this knowledge either before or during the course of their work.

Consistency checking

To assess agreement between appraisers, we will complete a Kappa analysis after appraising 20% of eligible studies. If the Kappa score is greater than 0.8, we will appraise the remaining studies. If the Kappa score is less than 0.8, we will discuss conflict literature to re-evaluate how each team member appraises studies. We will complete a second Kappa analysis on 20% of remaining eligible studies, and we anticipate this second analysis will yield a score greater than 0.8. We plan to require higher agreement during critical appraisal than screening because no further evaluation of studies occurs after appraisal.

Data coding strategy

We will extract plant species studied, pollinators identified, and methods used to identify pollinators. Rarely do flowering plants rely on a single pollinator, and correspondingly, we will extract taxonomic information provided for all pollinators identified within a study. We will use the framework established in Inouye et al. (1994) to classify methods used to identify pollinators by what aspect of pollen movement they observe. Pollination by animals has three stages: pollen removal from anthers, pollen transport by animal, and pollen deposition onto stigmas (Ne’eman et al. 2010). No single method can identify how animals pollinate, and combining methods can be challenging to undertake in a single study. We will classify each method by the pollination stage under study. If there are multiple studies for a given plant species and they studied different stages, we can combine this data to identify if certain pollinators served all stages of pollination. Alternatively, if multiple studies of the same plant examined the same stage, we can determine if their inferences coalesce around a common pollinator group. We will compile our database by entering data into a custom form developed with R Shiny.

Meta-data to be coded

We will extract and compile bibliometric and ancillary data into a separate database. Bibliometric data includes study title, author, and publication year. We will document the original source of studies as they are found across multiple types of literature, and we will record publication titles if available. Ancillary data includes details concerning study design, location, and populations. We will classify studies as observational trials or paternity testing. Observational trials record pollen removal or deposition from single flowers by animals and we will record the number of plants observed. For those involving paternity testing, we will record the number of molecular markers used, number of seeds and parent plants included, and effective population size (Ne) observed. We will record study locations and use this data to classify how much of the species range was examined. Inferences about pollinators are more reliable if the study captures more of the plant's geographic range, and this is especially true for wide-ranging species. We will use this data to assign confidence in pollinator assignments. We will record the total number of populations and number of individuals studied because population size directly affects the type and number of floral visitors. We will record calendar dates for field work, and we will compile our database by entering data into a custom form developed with R Shiny.

Consistency checking

Two people (BC and KGV) will independently extract and code study, bibliometric, and ancillary data. We will check and discuss discrepancies between the two sets of databases prior to combining them into a single set of databases for synthesis. This approach will ensure that data was extracted and checked by two team members.

Type of mapping

We (BC and RG) plan to use regression and ordination to interrogate our map database because these methods reflect the underlying nature of the relationships we seek to map. We anticipate many imperiled plants share pollinators, and ordination can group plants by their pollinators. Because resource biologists are our end-users, we need to characterize the quality of our database to allow biologists to accommodate for biases and uncertainty in their decision-making processes. Consequently, we plan to map our ancillary data using a combination of descriptive statistics and ordination to characterize common study designs, locations, and populations, pinpointing strengths and biases in the map database.

Narrative synthesis methods

BC, RG, ESK, and PMJ will synthesize databases for plant-pollinator interactions, study appraisal, and ancillary data using descriptive statistics. We will present summary statistics and plots with textual analysis to explain taxonomic trends among pollinators servicing listed plants.

Knowledge gap identification strategy

BC, RG, ESK, and PMJ aim to address three knowledge gaps: study methods, geographic patterns in plant-pollinator data, and USFWS recovery concerns. To identify gaps in study methods, we will use descriptive statistics to summarize data from study appraisal. Pollination consists of three stages—removal, transport, and deposition. Data from study appraisals will determine the most and least explored stages of pollination. BC and RG will use spatial regressions of species range to identify geographic gaps in plant-pollinator data. We expect plants with wide ranges are less studied than those from plants with narrow ranges as it is logistically easier to study pollinators in small, clustered populations. We will convert qualitative data on study locations and species range to GPS coordinates and then spatially regress geographic data to identify knowledge gaps based on species range. We will correct regressions for phylogenetic correlations if needed (Braga et al. 2018). ESK and PMJ will complement these regressions with USFWS recovery initiatives to identify biases in study locations due to funding access. USFWS can provide the most direct recovery actions for plants and pollinators occurring within its network of wildlife refuges. To guide conservation and recovery efforts, ESK and PMJ will identify pollinator needs for listed plants found in refuges within USFWS Region 4. They will also use this data to locate knowledge gaps about imperiled plants and pollinators on USFWS refuges.

Demonstrating procedural independence

BC and KGV have authored three publications that may be considered in this systematic map. If these publications are retrieved through searches, other team members (ESK and PMJ) will screen these studies for inclusion and critically appraise these studies as a part of the mapping process.

Competing interests

The authors declare no competing interests.

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Author’s contributions
BC, KGV, and RG conceptualized the map question. BC, BB, and PH developed protocols to search and screen literature and produced search terms. PH and BB completed pilot searches. BC, KGV, and RG developed protocols to extract and synthesize data from eligible studies. EK and PMJ contributed to protocols to critically appraise studies and identify knowledge gaps using ancillary data. BC wrote the first draft of this protocol with input from KGV, BB, EK, PMJ, and RG. All authors contributed to revisions.

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