

¹ Metagenomic Barcoding of Pollen Loads Offers Insights on the
² Foraging Patterns of Queen Bumble Bees

³ Reed Clark Benkendorf^{*1,2}, Jane E. Ogilvie ³, Emily J. Woodworth ^{1,2}, Sophie Taddeo ^{1,2}, Paul J. CaraJ

⁴ **Abstract**

⁵ .
⁶ 1) DNA Barcoding has been remarkably successful in nearly all kingdoms of life and has allowed for the
⁷ rapid analysis of ecological assemblies. Successful DNA barcoding in the plant kingdom has been more
⁸ difficult than other kingdoms. Due to this understanding plants in ecological contexts and understandings
⁹ of their synecology in some instances may begin to lag behind other kingdoms. 2) Here we utilize hyb-
¹⁰ seq, museum studies, and species distribution modelling, to detect the plant species present in pollen
¹¹ loads collected from Queen Bumble Bees. 3) By utilizing Species distribution modelling we allow for one
¹² to process hyb-seq data, create user specified sequence databases which may use MORE ACCURATE
¹³ alignment algorithms on personal computers over realistic time periods. 4) We show that hyb-seq using
¹⁴ the Angiosperms 353 probes, which are currently being used in the largest ever plant systematic endeavor,
¹⁵ offers significant promise to metagenomic approaches in real world scenarios. 5) We conclude that these
¹⁶ probes offer promise for the identification of plant tissue in both single sample, and metasample contexts.

¹⁷ **1 | INTRODUCTION**

¹⁸ < *UTILITY OF MASS IDENTIFICATION OF MATERIAL - FRIENDLY STAGE SET* >

¹⁹ < *ISSUES AND COSTS WITH IDENTIFYING MATERIAL TO SPECIES* > many spp defined by inter-
²⁰ actions (<;

²¹ The inability to reliably identify plants down to species can limit our understanding of ecosystem func-
²² tion and interactions. This is especially true for genera where the species are well defined based upon

*Correspondence: reedbenkendorf2021@u.northwestern.edu

23 ecological and behavioral rather than morphological properties, and hence can serve as key habitat bioindi-
24 cators(e.g. different species of Sagebrush- *Artemisia* L., Willows - *Salix* L., and Sedges - *Carex* L.) (Gage
25 & Cooper (2013)). The lack of species level data can hinder our understanding of the breadth of habitat
26 which some species occupy, and the interactions they have with other species (CITE). Current methods to
27 ameliorate this situation include: ignoring these ecologically relevant levels of detail, revisiting plots as diag-
28 nistic material becomes temporally available, assistance from taxonomic specialists, or the use of barcoding
29 or other molecular techniques.

30 The identification to species is often mired by lack of diagnostic characters (e.g. flowers, fruits, roots or
31 combinations thereof), an increasing lack of taxonomic experts (Hebert *et al.* (2003)) and increasingly the
32 description of cryptic species (Janzen *et al.* (2017), Oliver *et al.* (2009)). And revisiting field sites to identify
33 material using morphological or chemical approaches, can be resource intensive and often does not work.

34 < BARCODING HAS ITS LIMITS CURRENTLY BUT THERE IS POTENTIAL >

35 Recently barcoding (the identification of a sample from a single organism *e.g.* a piece of leaf), and metabar-
36 coding (the identification of a sample containing a mix of organisms *e.g.* soil), have shown considerable
37 promise in all Kingdoms of life (Ruppert *et al.* (2019)). With plants the identification of members of certain
38 clades using barcoding has been quite successful (REF), whereas with other clades results have been elusive
39 (Liu *et al.* (2014), Group *et al.* (2011), Coissac *et al.* (2012)), however metabarcoding incurs additional
40 challenges to those which exist for the currently implemented barcodes (Li *et al.* (2015), Kress & Erickson
41 (2007), Group *et al.* (2009), Coissac *et al.* (2012)). Particular challenges with barcodes include the uti-
42 lization of high-copy number sequences are associated with their rates of divergence, gene tree conflict, and
43 hybridization (Coissac *et al.* (2016), Fazekas *et al.* (2009)). Particular challenges with the utilization of high-
44 copy number sequences are associated with their rates of divergence, gene tree conflict, and hybridization
45 (Coissac *et al.* (2016)).

46 < WHAT NOVEL APPROACES IN ADDITION TO A353 ?>

47 Currently the largest plant systematic endeavor ever undertaken, the Kew Plant and Fungal Tree of Life
48 (PAFTOL), is approaching completion (Baker *et al.* (2021)). This data set will contain Hyb-Seq data from
49 at least one species representing each genus in the plant kingdom using the popular A353 probes (Baker *et*
50 *al.* (2021)), resulting in over 14,000 represented species. These publicly available data serve to provide a
51 taxonomically comprehensive backbone for plant metabarcoding. Data from the 10kP project, which seeks
52 to develop reference genomes from a phylogenetically diverse suite of plants will contribute many more
53 records upon it's intended completion, now slated to be by 2030, similar projects which seek to sequence

54 high amounts of genomes in regions e.g. the ‘Darwin Tree of Life’ are being undertaken which will contribute
55 data applicable to enormous spatial domains (Cheng *et al.* (2018), Life Project Consortium *et al.* (2022),
56 Lewin *et al.* (2022)). These data will promote the ability to apply metabarcoding to resolve a diversity
57 of questions relevant to theoretical and applied ecology (cite). However, the application of metabarcoding
58 still face challenges relating to the enormity of the genomic data sets and the computational power required
59 to process sequence data. Herein we have resolved major components of the problems of identifying plant
60 material without diagnostic morphological character states using the Angiosperms353 (A353) Hyb-Seq probes
61 (Johnson *et al.* (2019)), and custom species sequence databases derived via species distribution modelling,
62 and temporal filtering.

63 < THIS P – WHAT WE DO TO TEST THIS OUT >

64 To increase the quality of metabarcoding results in plants, we suggest reducing the number of possible
65 plant species candidates by generating user selected sequence databases relevant to the the study region
66 and its ecological characteristics (CITE !?). To achieve this goal, we first create a list of candidate species
67 using digital collections gleaned from herbaria, survey work, and citizen science (e.g. iNaturalist), from
68 a region exceeding the study area. To these candidate species, modelling approaches - such as logistic
69 regression, may be used to identify taxa which warrant further exploration e.g. modelling to determine
70 their possibility of presence in metabarcoding samples. We then use species distribution models to create
71 potential distribution maps for the candidate species to limit the impact of spatial and taxonomic biases in
72 the species list and account for spatial variations in niche availability throughout the study area. Species
73 distribution models (SDM’s) examine the ecological conditions associated with known occurrence of a species
74 to identify where else in the study area might suitable habitats be found. This approach has the additional
75 benefit of greatly reducing the size of a sequence database, which allows for the usage of genomic size
76 data on personal computers. This can also significantly reduce processing time, particularly as as most
77 next-generation sequence data is deposited as raw-sequence reads.

78 Considerable amounts of species interactions vary along time (CaraDonna *et al.* (2021)). For the tropics
79 and subtropics, contrasts in the flowering periods of many plant species, can provide an additional filter for
80 identifying material in many metagenomic samples (Janzen (1967), Newstrom *et al.* (1994)). In temperate
81 regions, pollination interactions also vary temporally (CaraDonna *et al.* (2017)), however the overall shorter
82 extent of the active growing season in these systems results in the presence of few to any natural breaks
83 in these systems which reduces the utility of these to operate as filters in the post-processing of sequence
84 matches. Nonetheless, we work through a process which seems applicable to the tropics and subtropics to
85 utilize the temporal dimension for classifying sequencing results.

86 To test these metagenomic and informatics approaches to determine whether the foraging record of Queen
87 Bumble Bee's is consistent across direct observations and the pollen record, an incongruity in several floral
88 visitation networks involving smaller bodied fauna (Barker & Arceo-Gomez (2021), Zhao *et al.* (2019),
89 Alarcón (2010)). The assessment of the plant species compositions of pollen is a desired results, with
90 several applications, and numerous complications (Poronon *et al.* (2017), Bell *et al.* (2017), Sickel *et al.*
91 (2015), Bell *et al.* (2019), Suchan *et al.* (2019), Johnson *et al.* (2021)). The two foraging phases of
92 the Queen Bumble Bee life cycle is essential to 1) increase their weight before diapause, 2) increase their
93 ovary weights while establishing their recently found nests, both of these time periods represent potential
94 demographic bottlenecks in bumble bee populations (Sarro *et al.* (2022)). Bumblebees are one of the
95 only groups of insects with unequivocal quantitative evidence for numerous populations declines, while
96 simultaneously serving as the most effective pollinators in temperate montane ecosystems (Cameron & Sadd
97 (2020), Goulson *et al.* (2008), Williams (1982), Colla *et al.* (2012), Bergman *et al.* (1996), Bingham &
98 Orthner (1998)). *Heterogeneous montane ecosystems represent one of the most ecologically resilient areas*
99 *in the temperate and oftentimes offer the sole potential refugia for multiple dimensions of biodiversity under*
100 *climate change, whilst simultaneously experiencing the most abrupt changes in mean annual temperature*
101 *(CITE). An immediate understanding of how to manage previously overlooked keystone insect species, such*
102 *as bumble bees, is essential if these refugial ecosystem services are to be utilized while maintaining their*
103 *current species richness.*

104 2 | METHODS

105 Study System & Field Work

106 Observations and sample collection was conducted at The Rocky Mountain Biological Laboratory (RMBL;
107 38°57.5" N, 106°59.3" W (WGS 84), 2900 m.a.s.l.), Colorado, USA (APPENDIX 1 for site information). Pol-
108 linator observations of Bombus Latreille spp. (Apidae Latreille) were conducted from June - August of 2015
109 in six study sites characterized by high-montane/subalpine Parkland vegetation communities. Observations
110 of Bombus foraging took place for one hour at each field site in three 100m transects, where all flowers were
111 also counted and placed into abundance bins. Corbiculae loads were, non-lethally, collected once from all
112 Queen individuals encountered.

113 **2.1 | Spatial Analyses**

114 **2.1.1 Candidate Species** We downloaded from the Botanical Information and Ecology Network ‘BIEN’
115 (Maitner (2022)) all records adjacent to the field sites to develop an ecologically relevant list of vascular
116 plant species, with expected biotic pollination, which may be present at the study area . We then generated
117 Species Distribution Models (SDMs) to predict their distribution throughout the study area. These maps of
118 potential distribution served as a reference to reduce the list of species to include in the genomic sequence
119 databases.

120 In order to minimise the number of species for which SDM’s were to be generated, BIEN was queried at
121 a distance of up to 100km from our study area and all plant species records were downloaded. ***In order***
122 ***to emulate the stochasticity of botanical collecting, this data set was bootstrap re-sampled 250***
123 ***times, with 90% of samples selected, to create a testing data set.*** The median of the logistic
124 regression assessing the probability of occurrence of a species record as a function of distance from the
125 study area was used as a threshold distance, under which, to include species as candidates for distribution
126 modelling.

127 **2.1.2 Distribution Modelling** We used all occurrence records from BIEN ($n = 23,919$) within a 50km
128 border of the Omernik level 3 ecoregion, which includes the study area (*No. 21 “Southern Rockies”*) to
129 construct the species distribution model (Omernik (1987)). These records were copied into two, initially
130 identical, sets, one for generating machine learning models (ML; Random Forest, and Boosted Regression
131 Tree’s), and the other for Generalised Linear (GLM) and Generalized Additive Models (GAM) (Barbet-
132 Massin *et al.* (2012)). **Ensembled predictions have been shown to outperform their constituent**
133 **models, on average, and to reduce the ecological signal to the analytical noise of individual runs**
134 **(Araujo & New (2007)). No single method of producing SDMs has been shown to universally**
135 **outperform others when faced with a large and diverse number of applications, in our case a**
136 **great number of species with differing biologies and ecologies (Elith* *et al.* (2006), Qiao *et al.***
137 **(2015)). In the spirit of these findings, multiple families of models, which can be generated**
138 **together as they have similar requirements regarding the number and ratios of Presence to**
139 **Absence records were ensembled together (Barbet-Massin *et al.* (2012)).**

140 We then generated 4,029 absence points , locations where the focal taxon is anticipated missing, through a
141 random stratification of 19% of the land cover in the area and included them in (BLM CITATION - need
142 appropriate format for journal). To achieve a larger absence data set, we generated 1,000 pseudo-absence
143 records for each taxon by randomly selecting coordinates located at least 10km away from an occurrence

¹⁴⁴ record. For ML models, these pseudo-absences were reduced so that the ratio of presence to absence records
¹⁴⁵ were balanced (Barbet-Massin *et al.* (2012)). To achieve this, we removed absence records inside of 10% of
¹⁴⁶ the mean sample value of the presence records; the required number of absence records were then randomly
¹⁴⁷ sampled.

¹⁴⁸ We used 26 environmental variables at 30m resolution to predict the potential distribution of each species,
¹⁴⁹ six related to climate, five soil, four topographic, four related to cloud cover, with the remaining reflecting
¹⁵⁰ assorted abiotic parameters (Wilson & Jetz (2016), Wang *et al.* (2016), Hengl *et al.* (2017), Robinson *et al.*
¹⁵¹ (2014)) (*APPENDIX 6*). **These publicly available data sets, were selected as they** For linear
¹⁵² regression models these predictors underwent both *vifstep* (theta = 10, max observations = 12,500) and
¹⁵³ *vifcor* (theta = 0.7, max observations = 12,500) to detect highly correlated variables, and collinear features
¹⁵⁴ were removed leaving 16 variables (Naimi *et al.* (2014)).

¹⁵⁵ Modelling: Random Forest and Boosted Regression Trees, were sub sampled with 30% test and two replicates
¹⁵⁶ each before weighted ensemble based on True Skill Statistics (tss) (Naimi & Araujo (2016)). Generalised
¹⁵⁷ linear models (GLM) and Generalised additive models (GAM) with 30% sub sampling and three replicates
¹⁵⁸ each were also ensembled using the tss (Naimi & Araujo (2016), @). TSS was chosen as the ensemble
¹⁵⁹ criterion as it has been shown to work across a range of species occurrences prevalence (Allouche *et al.*
¹⁶⁰ (2006)). The results of these models were extracted on a cell-by-cell basis to a polygon feature derived from
¹⁶¹ a minimum-spanning tree which encompasses the study sites, and species from either ensemble with greater
¹⁶² than 50% mean habitat suitability across all cells were considered present for further purposes (Prim (1957)).

¹⁶³ 535 species were modelled using Generalized Linear Models and Generalized Additive Models. 534 species
¹⁶⁴ were modelled using Random Forest and Boosted Regression Trees. To evaluate the accuracy of the species
¹⁶⁵ distribution models, additional presence records from GBIF (n = 61,789), and AIM (n = 12,730) were used as
¹⁶⁶ test and training sets (n = 74,519) for logistic regression (CITE AIM AND Occdownload Gbif.Org (2021)).
¹⁶⁷ Additional novel absence records were generated from the AIM data set to create a data set where each
¹⁶⁸ species has balanced presence and absences. 11 or more paired presence and absence records were required
¹⁶⁹ for this testing, resulting in 334 species being included in the logistic regression (Mdn = 110.0, \bar{x} = 223.1,
¹⁷⁰ max = 1568 record pairs used) with a 70% test split (Kuhn (2022)).

¹⁷¹ 2.2 | Molecular Lab Work

¹⁷² All lab work was carried out at The Daniel F. and Ada L. Rice Plant Conservation Science Center at the
¹⁷³ Chicago Botanic Garden, Glencoe, Illinois, U.S.A.

174 **2.2.1 | Reference Plant Library Generation** Using five years of observational data on *Bombus* Queen
175 Bee foraging at these studies sites, we identified the plant taxa most frequently visited by Queens across
176 all years. We sequenced the 12 most commonly visited taxa twice using samples from one site within the
177 Gunnison River Drainage and one individual from another population. In addition, for any of these 12 focal
178 species which did not have a congener pair in this filtered sample, we included a congener - or a species from
179 a closely related genus to serve as an outgroup. We also sequenced another 15 abundant taxa commonly
180 visited by *Bombus* workers, based on the aforementioned data set (*APPENDIX 4*).

181 **2.2.2 | Plant Genomic DNA Extraction** Plant genomic DNA was isolated from ~ 1 cm² of leaf tissue
182 from silica-gel dried or herbarium material using a modified cetyltrimethylammonium (CTAB) protocol
183 (Doyle & Doyle (1987)) that included two chloroform washes. DNA was quantified using a Nanodrop 2000
184 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and Qubit fluorometer (Thermo Fisher Scientific).

185 **2.2.3 | Pollen Genomic DNA Extraction** Pollen genomic DNA was extracted from corbiculae using a
186 CTAB based protocol modified from Lahlamgiah et al. and Guertler et al. (2014, 2014). A SDS extraction
187 buffer (350µL , 100mM Tris-HCl, 50 mM EDTA, 50 mM NaCl, 10% SDS v/v., pH 7.5) was added followed by
188 vortexing to allow dissolution of corbiculae. Pollen grains were then macerated with Kontes Pellet Pestles,
189 and the tip of these washed with 130 µL of the SDS extraction buffer, samples were then incubated for
190 1 hour at 30°C. This was followed by the addition of 10% CTAB solution (450ul, of 20 mM Tris-Cl pH.
191 8.0, 1.4 M NaCl, 10 mM EDTA pH 7.5, 10% CTAB, 5% PVP, ~85% Deionized water) and RNase (10
192 uL of 10 mg/mL) and samples were incubated for 40 minutes at 37°C, on heat block (Multi-Blok, Thermo
193 Fisher Scientific, Waltham Massachusetts) set to 40°C. After 20 minutes incubation, Proteinase K (15 µL of
194 20mg/ml) and DTT (12.5 µL of 1M in water) were added, and the samples were further incubated at 60°C
195 for 1 hour. Samples were then incubated overnight at 40°C. 500 µL of Phenol-Chloroform-Isoamyl alcohol
196 (25:24:1) were added, vortexed, and centrifuged at 10,000 rpm for 10 minutes and the aqueous phase was
197 pipetted to a 1.5 ml centrifuge tube.

198 To precipitate the DNA, chilled Isopropyl alcohol & 3 mM Sodium acetate (5:1) equivalent to 2/3 of the
199 volume of sample were added, with 1 hour of chilling at -20°C, followed by 10 minutes of centrifuging at
200 13,000 rpm. The supernatant was pipetted to a new 1.5 ml centrifuge tube, and 70% EtOH (400 µL) were
201 added before chilling at -20°C for 20 minutes followed by centrifugation at 13,000 rpm for 10 minutes. Both
202 tubes were then washed with 75% EtOH (400 µL), inverted, centrifuged at 13,000 rpm for 4 minutes, and
203 the solution discarded, then washed with 95% EtOH (400 µL) , inverted, centrifuged at 13,000 rpm for 4

204 minutes, and the solution discarded. Pellets were dried at room temperature overnight before resuspension
205 in Nuclease free H₂O. Extractions were assessed using a Nanodrop 2000 (Thermo Fisher Scientific) and
206 Qubit fluorometer (Thermo Fisher Scientific). DNA extracts were then cleaned using 2:1 v./v. Sera-Mag
207 beads (Cytiva, Little Chalfont, UK) to solute following the manufacturer's protocol, eluted in 0.5x TE, and
208 the eluent allowed to reduce by half volume in ambient conditions. DNA was quantified using a Qubit
209 fluorometer.

210 **2.2.4 | Fragmentation, Library Preparation & Target Enrichment** Library preparation was per-
211 formed using the NEBNext Ultra II FS-DNA Library Prep Kit for Illumina (New England BioLabs, Ipswich,
212 Massachusetts, USA) using slightly modified manufacturers recommendation. Fragmentation was performed
213 at ½ volume of reagents and ¼ enzyme mix for 40 minutes at 37°C, with an input of 500 ng cleaned DNA.
214 Adapter Ligation and PCR enrichment were performed with ½ volumes, while cleanup of products was
215 performed with ½ volume of SPRI beads (Beckman Coulter, Indianapolis, Indiana, USA) and recommended
216 volumes of 80% v./v. ethanol washes. The exception was the herbarium specimens which were not frag-
217 mented and only end repaired, with similar library preparation of all samples. Products were analysed on
218 4% agarose gels, and a Qubit fluorometer. Libraries were pooled and enriched with the Angiosperms 353
219 probe kit V.4 (Arbor Biosciences myBaits Target Sequence Capture Kit) by following the manufacturer's
220 protocol and Brewer *et al.* 2019. Sequencing was performed using an Illumina mi-Seq with 150-bp end reads,
221 (NUSeq Core, Chicago, Illinois).

222 **2.2.5 | Computational Processes and Analyses.**

223 **2.2.5.1 | Reference Library Data Processing** Sequences were processed using Trimmomatic, which
224 removed sequence adapters, clipped the first 3 bp, discarding reads less than 36 bp, and removing reads
225 if their average PHRED score dropped beneath 20 over a window of 5 bp (Bolger & Giorgi (2014), Tange
226 (2021)). Contigs were generated using HybPiper using target files created by M353 (Johnson *et al.* (2016),
227 McLay *et al.* (2021)).

228 **2.2.5.2 | Sequence Identification** A custom Kraken2 database was created by downloading represen-
229 tative species of each genus indicated as being present in the study area by the spatial analyses from the
230 Sequence Read Archive (SRA) NCBI (Wood *et al.* (2019)). These sequences were processed in the same
231 manner as our novel sequences . The Kraken2 database was built using default parameters. Kraken2 was
232 run on sequences using default parameters (*APPENDIX 5*). Following Kraken2, Bracken was used to clas-

233 sify sequences to terminal taxa (Lu *et al.* (2017)). Results from both Kraken2 and Bracken, results were
234 reclassified manually to identify terminal taxa. For example, when only a single species of a genus was known
235 in the study area, but our database used a representative of another taxon in the genus, this species was
236 coded as the result. The re-coding of sequences from another representative species for the genus to the sole
237 RMBL representative allowed the identification of XX & % more species.

238 **2.2.5.3 | Identification of Sequence Matching Loci** A local NCBI database was built using the same
239 processed novel and downloaded sequences (Camacho *et al.* (2009)).

240 **2.2.5.4 | Morphological Pollen identification**

241 To develop a reference library of pollen grains which may be present in corbiculae loads, an image reference
242 collection of fuchsin-jelly stained (Beattie (1971)) slides was assembled from slides previously prepared by the
243 authors (n = 21), and other researchers (n = 38) (Brosi & Briggs (2013)). Using five years of observational
244 data on *Bombus* Queen Bee foraging at these studies sites (Ogilvie unpublished), as well as the Vascular
245 Plant Checklist (Frase & Buck (2007)), an additional 62 voucher slides for species were prepared and imaged
246 at 400x (Leica DMLB, Leica MC170 HD Camera, Leica Application Suite V. 4.13.0) from non accessioned
247 herbarium collections to supplement the number of species and clades covered (Appendix 3).

248 We used Divisive Hierarchical Clustering techniques to determine which plant taxa were distinguishable via
249 light microscopy, and to develop a dichotomous key to pollen morphotypes. Ten readily discernible categorical
250 traits were collected from each specimen in the image collection. These traits were transformed using Gower
251 distances, and clustered using Divisive Hierarchical clustering techniques (Maechler *et al.* (2022)). Using
252 the cluster dendrogram, elbow plot, and heatmaps (Hennig (2020)), of these results morphological groups
253 of pollen which could not be resolved via microscopy were delineated, and a dichotomous key was prepared
254 (APPENDIX NO.). This key was then used to identify the pollen grains sampled from corbiculae loads to
255 morphotypes in a consistent manner. To prepare the pollen slides from corbiculae, all corbiculae loads were
256 broken apart and rolled using dissection needlepoints to increase heterogeneity of samples. *Cerca* 0.5mm²
257 of pollen was placed onto a ~4mm² fuchsin jelly cube (Beattie (1971)) atop a graticulated microscope
258 slide, with 20 transects and 20 rows (400 quadrants) (EMS, Hartfield, PA). The jelly was melted, with
259 stirring, until pollen grains were homogeneously spread across the microscope slide. Slides were sealed with
260 Canada Balsam (Rublev Colours, Willits, CA) followed by sealing with nail polish; all samples are noted in
261 APPENDIX 3. To identify the pollen present in corbiculae loads, light microscopy at 400x (Zeiss Axioscope
262 A1) was used. In initial sampling in three transects, each pollen grain was identified to morphotype and

263 counted; an additional two transects were scanned for morphotypes unique to that slide, if either transect
264 contained an unique morphotype than all grains in that transect were also identified and counted. Subsequent
265 to the first round of sampling, non-parametric species richness rarefaction curves (Oksanen *et al.* (2022)),
266 and non-parametric species diversity rarefaction curves were used to assess the completeness of sampling
267 (Chao *et al.* (2014), Hsieh *et al.* (2020)). Slides not approaching the asymptote of the rarefaction curve
268 were then re-sampled, and analysed iteratively for up to a total of seven transects *APPENDIX 2*.

269 **2.3 | Temporal Analyses**

270 To estimate the duration of dates in which plant species were flowering weibull estimates of several pheno-
271 logical parameters all spatially modelled taxa were developed (Belitz *et al.* (2020), Pearse *et al.* (2017)).
272 Only BIEN records which occurred in the Omernik Level 4 Ecoregions within 15km of the study area ($n = 5$
273 Level 4 Ecoregions, or conditionally 6 if enough records not be found in the nearest 5), and which were from
274 herbarium records were included. To remove temporally irrelevant herbarium records, i.e. material collected
275 during times which flowering is impossible at the study area due to snow cover, we used the SnowUS data
276 set (Iler *et al.* (2021), Tran *et al.* (2019)) from 2000-2017 was analyzed for the first three days of contiguous
277 snow absence, and the first three days of contiguous snow cover in Fall. Herbarium records after the 3rd
278 quantile for melt, and the 1st quantile for snow cover of these metrics were removed. Species with > 10
279 records had their weibull distributions generated for the date when 10% of individuals had begun flowering,
280 when 50% were flowering, and when 90% of individuals had flowered.

281 **2.4 | Floral Observations**

282 **3 | RESULTS**

283 **3.1 | Spatial Analyses**

284 [Table 1 about here.]

285 [Table 2 about here.]

286 The median (25.009 km) of the logistic regression assessing the probability of occurrence of a species record as
287 a function of distance from the study area was used as a threshold distance to include species for distribution
288 modelling. A 2-sample test for equality of proportions with continuity correction ($X^2 = 13.254$, df

²⁸⁹ = 1, p-value = 0.000136, 95% CI 0.04-1.00) was used to test whether more of the records located in the
²⁹⁰ broad ecological sites present at the field station, between the distance of the median (25.009 km) to the
²⁹¹ third quantile (ca 43.830 km) of the regression distance, where true presences at the field station. Including
²⁹² these records would have resulted in modelling an additional 222 species distributions of which 30 are true
²⁹³ presences these taxa were not modelled.

²⁹⁴ Across the entire spatial domain of modelling all ensembled models (n = 968) had an accuracy of 0.84 (95%
²⁹⁵ CI 0.8356 - 0.8443), kappa 0.68, p-value < 0.001, sensitivity = 0.80, specificity = 0.87, AUC = 0.92.

²⁹⁶ At the field site, of the 554 vascular plants with biotic pollination syndromes, the 493 ML ensembles accu-
²⁹⁷ rately predicted the presence of 362 (65.3%), incorrectly predicted the presence of 64 (11.6%), incorrectly
²⁹⁸ predicted 34 true presences (6.1%) as being absent, and correctly predicted the true absence of 33 (6.0%).

²⁹⁹ The balanced accuracy of the ensembled models is 0.627 (Sensitivity = 0.340, Specificity 0.914). Of the 554
³⁰⁰ vascular plants with biotic pollination syndromes, the 475 LM ensembles accurately predicted the presence
³⁰¹ of 286 (51.6%), incorrectly predicted the presence of 41 (14.3%), incorrectly predicted 93 true presences
³⁰² (16.8%) as being absent, and correctly predicted the true absence of 55 (9.9%). The balanced accuracy of
³⁰³ the ensembled models is 0.664 (Sensitivity = 0.573, Specificity 0.754). Of the 554 vascular plants with biotic
³⁰⁴ pollination syndromes in the flora 13 (2.3%) were in the Orchid family and 41 (7.4%) are non-natives, both
³⁰⁵ of which are restricted from the database, and can only reduce the number of true predicted presences by
³⁰⁶ roughly 10%.

³⁰⁷ At the six study plots, of the 117 plant species identified to the species level across the spatial extents of all
³⁰⁸ plots and duration of queen bee activity, the ML ensembles predicted the presence of 105 (89.7%) of them,
³⁰⁹ and LM ensembles 102 (87.2%). Of the missing species two (1.7%) are Orchids, six (5.1%) are non-native,
³¹⁰ and one (0.85%) is of contested taxonomic standing, all of which (7.65%) are restricted from the initial query
³¹¹ database.

³¹² 3.2 | Microscopic Pollen identification

³¹³ Using the fuchsin jelly preparation and light microscopic analyses of grains and scoring of 10 character
³¹⁴ states resulted in the establishment of 28 morphotypes which grains could be classified into. APPENDIX
³¹⁵ 7. 60 samples were counted and based on rarefaction **had over % of expected morphotypes found**
³¹⁶ (morphotype richness, $\bar{x} = 4.5$, Mdn = 4, min = 1, max = 9), all samples had expected morphotype diversity
³¹⁷ reach the asymptote APPENDIX 8. The number of counted pollen grains in each sample range from (MIN
³¹⁸ - 16,293, $\bar{x} = 2788.685$, Mdn = 1453).

319

[Figure 1 about here.]

320 3.3 | Metabarcoding Pollen identification

321 54 corbiculae loads had DNA extracted and underwent various steps towards hyb-seq, in the end a total of
 322 44 corbiculae samples were sequenced, 7,752,353 reads were recovered from sequencing. The number of reads
 323 per sequence varied widely (range = 76 - 508,795, $\bar{x} = 176,189.8$, Mdn = 138,395). Of the possible 353 loci,
 324 the number which were recovered from each sample, and informative to BLAST were range = 24 - 353, $\bar{x} =$
 325 305.5, Mdn = 331. The number of reads per loci from across all samples had a range of 178 - 506,653, $\bar{x} =$
 326 20,688, Mdn = 12,616.

327 APPENDIX X Reads Per Loci.

328 After trimming 7,865,680 sequences remained. 10,682,538 reads were matched using Kraken, of the reads
 329 classified by Kraken 10,160,768 reads were matched using Bracken, of the reads classified by Kraken 7,302,876
 330 reads were matched using BLAST.

331 Based upon subjective review of the three classifiers **APPENDIX X MOLECULAR NETWORKS -**
 332 **3 DIFFERENT ONES**, BLAST was chosen as the classification method which yielded the most probable
 333 results, and it's values were used for all subsequent analyses.

334 3.4 | Temporal Analyses

335 The first date of modeled snow melt in the Gothic area ($n = 17$, $\bar{x} = 137.9$, Mdn = 135, 3rd quantile = 151),
 336 and the first date of a consistent winter snow base ($n = 17$, $\bar{x} = 299.9$, Mdn = 300, 1st quantile = 291) from
 337 2000-2017, were used as delimiters for the inclusions of herbarium records in modelling. Of the **500** species
 338 predicted likely present in the area via logistic regression, 332 species (64.4%) with more than 10 records in the
 339 focal level 4 ecoregions ($\bar{x} = 35.01657$, Mdn = 35, max = 96) had weibull estimates calculated, an additional
 340 56 species (11.2%) with enough contributing records from the “Sedimentary Mid-Elevation Forests”, a large
 341 ecoregion in general just beneath the elevation bands occupied by the five ecoregions around the study area
 342 had weibull estimates also calculated ($\bar{x} = 13.86885$, Mdn = 13, max = 24).

343 Only 58 of these 388 species ($n = 34.56897$, Mdn = 31) were able to be compared to plot based observational
 344 data from the long term (1974–2012) data set. Of these species relatively high accord was observed between
 345 the long-term ground truthed data set, and the modelled species. There was very strong evidence that the
 346 weibull estimates were positively associated with the observed onset ($r^2 = 0.72$, $p < 0.0001$, $\tau = 0.61$)

347 and peak ($r^2 = 0.70$, $p < 0.0001$, $\tau = 0.65$) of flowering, and that the number of herbarium samples had
348 a moderate effect on the estimates ($p = 0.004$ and $p = 0.034$ respectively). There was very strong evidence
349 that the weibull estimates had a positive association with the observed cessation of flowering ($r^2 = 0.4339$, p
350 < 0.0001 , $\tau = 0.489$), however there was no evidence that sample size had an effect ($p = 0.349$). There was
351 moderate evidence that the weibull estimates, with an effect of sample size, had a weak positive association
352 with the observed duration of flowering ($p = 0.0401$, $r^2 = 0.07$, $\tau = 0.17$).

353 [Figure 2 about here.]

354 3.5 | Floral Observations

355 The six sites were surveyed for a total of 52 hours from May 27-July 27. A total of 723 queen-pollen foraging
356 interactions were observed (range per bee species by week range = 1 - 18, $\bar{x} = 3.46$, Mdn = 2), with a
357 range of total observed interactions per bee species across this time period (min = 1, $\bar{x} = 59.08$, Mdn = 19,
358 max = 184). Plants varied widely in the number of interactions which they partook in with each species
359 of bee (range per plant species by week min = 1 - 20, $\bar{x} = 3.51$, Mdn = 2), with a range of total observed
360 interactions per plant species over this time period (min = 1, $\bar{x} = 20.26$, Mdn = 4, max = 141). The number
361 of plant species which bees were observed interacting with varied more narrowly (range = 1 - 18, $\bar{x} = 8$,
362 Mdn = 6).

363 A total of 66 corbiculae loads were collected from Bees, 64 of them from Queens.

364 3.6 | Integrated Observational, Molecular, and Palynological Network

365 ... get ...

366 4 | DISCUSSION

367 **overall stuff and things** We have demonstrated how Angiosperms533 data may be used for plant bar-
368 coding in a metagenomic context. This was exemplified in an ecologically realistic situation, centered both
369 in a context which serves as an immediate framework for land management studies, and which produced
370 fundamental natural history information. These data sets included closely related taxa in notoriously taxo-
371 nomically difficult clades (e.g. *Mertensia*, *Lupinus*). We utilized spatial and temporal approaches for creating

372 custom sequence databases which are readily applicable to any lab group with the capacity to perform next-
373 generation sequencing across the entirety of multiple continents. In conjunction with well designed and
374 implemented field based data collection we show how these methods may be widely used to test a variety of
375 hypotheses related to ecological interactions.

376 These results show that the overall results between **Bumble Bee ecology** observational and barcoding are
377 largely congruent. But that ... Future analyses of the long term data set... Global studies should aim to
378 corroborate ...

379 **Spatial ... & Timing** (filters!) - Fewer modelling runs for SDM's likely to be effective for determining
380 inclusion, elastic inclusion criteria per application. - The actual data set which was used for training and
381 testing all of the models incorporated into SDM's represented only roughly one quarter of the records
382 available for such purposes. We consciously chose to do this in order to showcase the possibility of this
383 approach working in less data rich areas. - Used fine scale flora to evaluate results of modelling, similar
384 approaches based on quick field surveys which do not require comprehensive floristic endeavors. - Timing
385 in other areas may suffer complications due to lacking as strong a signal as that induced by snowmelt, but
386 longer durations of flowering likely to help. - Sample size in phenology estimates had an effect which is likely
387 to be maintained across all results. - Regardless both show good agreement on flower onset, peak flowering,
388 and moderate agreement with flowering cessation. The disagreement in flowering cessation is perhaps due
389 to more microclimates which retain water, rather than microclimates which allow the early accumulation of
390 heat. - Number of botanists rapidly decreasing no hope for much more field based information or funding.

391 **Molecular ...** - PAFTOL project provide complete genus level reference data set for the kingdom -
392 Expected to work nearly immediately for DNA barcoding of whole plant material. - With work expected
393 to work well in meta contexts. - Harvest both variable loci, and variable flanking regions of loci for fine
394 taxonomic resolution - Potential for infra-specific inference (Wenzell *et al.* (2021), Loke *et al.*, Slimp *et al.*
395 (2021), Beck *et al.* (2021)).

396 **Final P** < bayesian framework > & < harvesting loci > = Possible Application at landscape scale.

397 **AUTHOR CONTRIBUTIONS:** R.C.B conducted botanical collections, conducted all molecular lab
398 work, lead all analyses, and writing. J.E.O conceived, designed, and conducted all ecological fieldwork,
399 assisted with analyses, and writing. E.J.W. prepared, imaged, and collected trait data on pollen reference
400 slides, and assisted with analysis of trait data and writing a dichotomous key. S.T. assisted with spatial
401 analyses and writing. P.J.C assisted with ecological analyses and writing. J.B.F. conceived, and designed all
402 lab work, analyses, and integration of approaches, assisted with writing, and secured funding for molecular

403 work.

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415 Cole for sharing the Angiosperm Phylogeny 4 colour palette.

416 **CONFLICT OF INTERESTS** The authors declare no conflicts of interest.

417 **PEER REVIEW** The peer review history for this document is available at ...

418 **DATA AVAILABILITY STATEMENT** The queries required to download all data used in this project
419 are located in.... All novel sequencing data are located at NCBI...

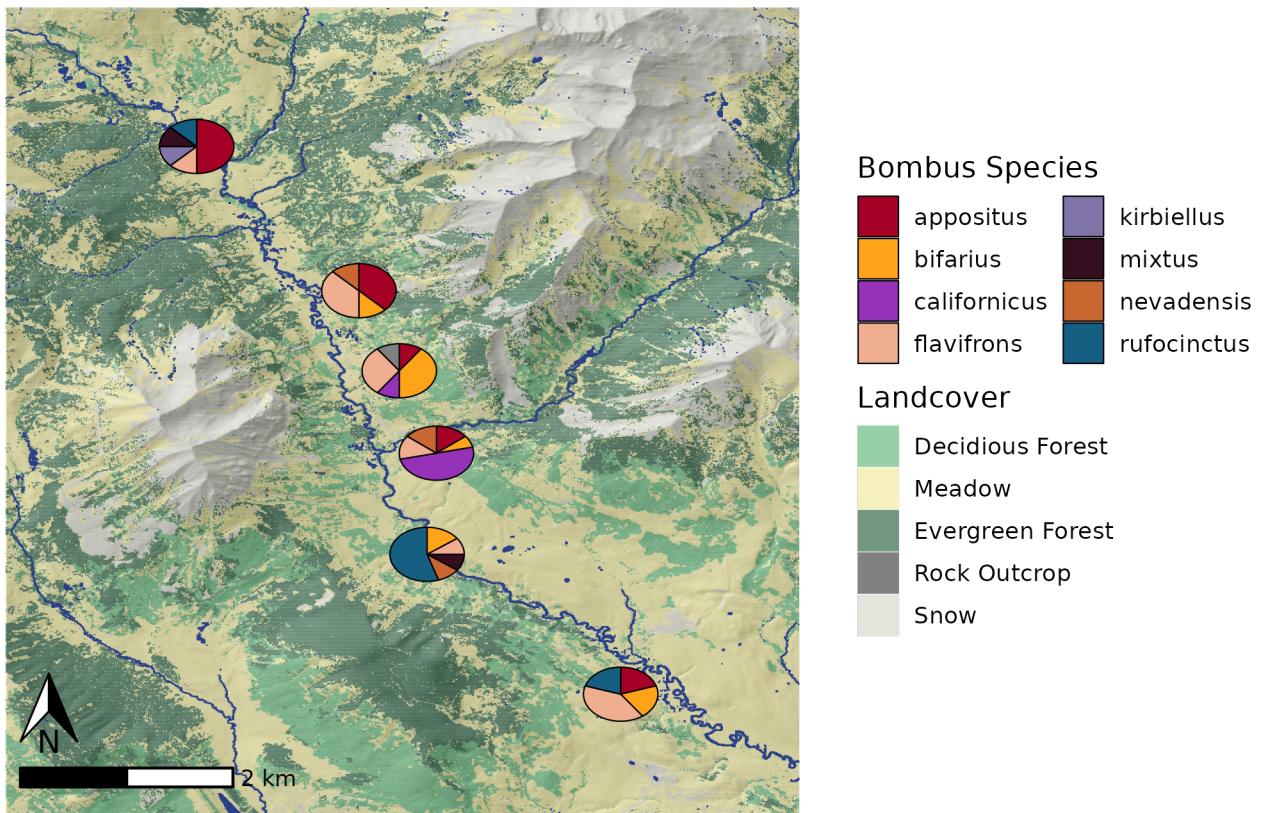
420 **ORCID**

421 Paul CaraDonna <https://orcid.org/0000-0003-3517-9090>
422 Jeremie Fant <https://orcid.org/0000-0001-9276-1111>
423 Jane Ogilvie <https://orcid.org/0000-0001-8546-0417>
424 Sophie Taddeo <https://orcid.org/0000-0002-7789-1417>

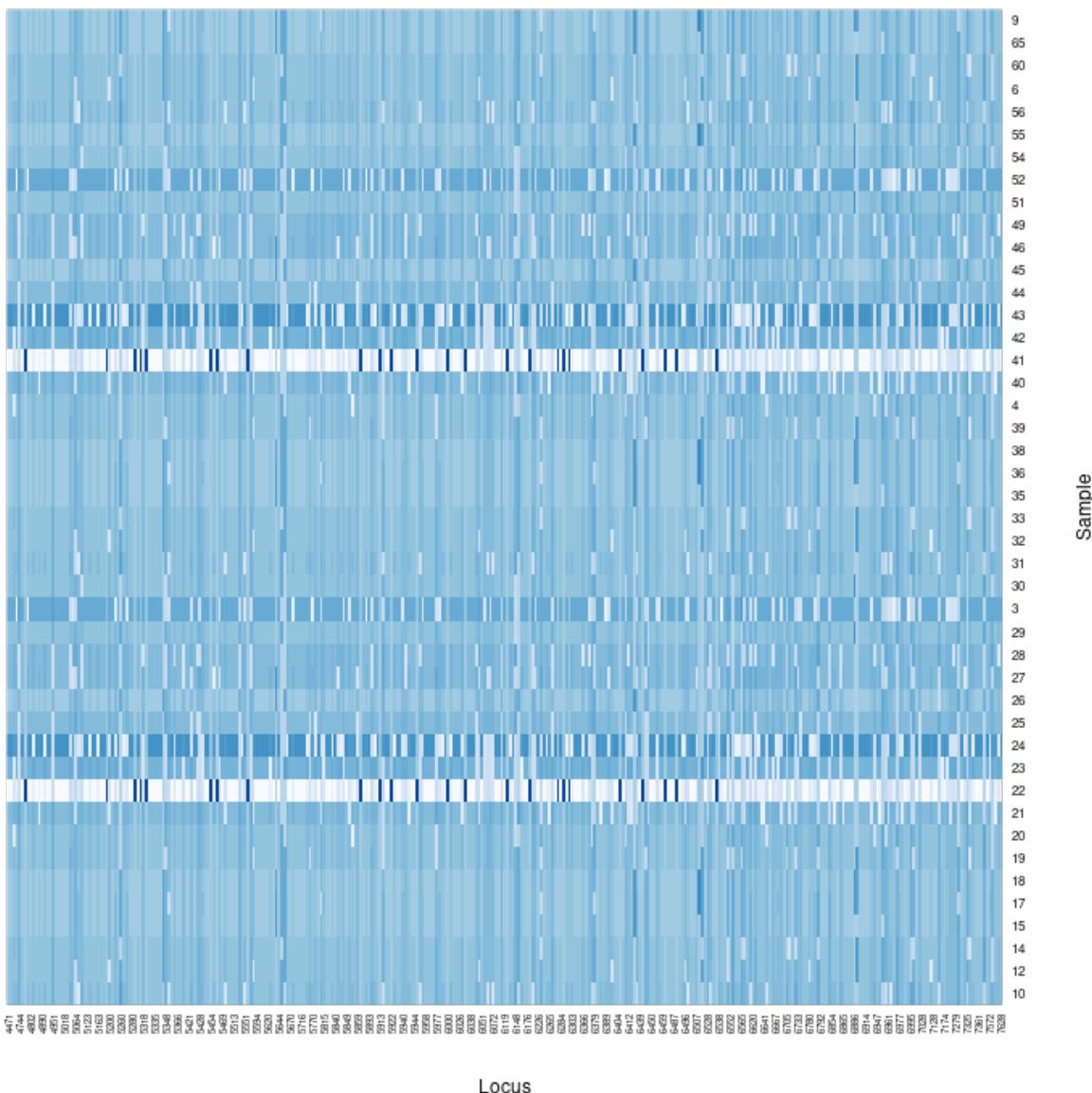
425 **References**

426 **Supporting**

Origins of Corbiculae Loads



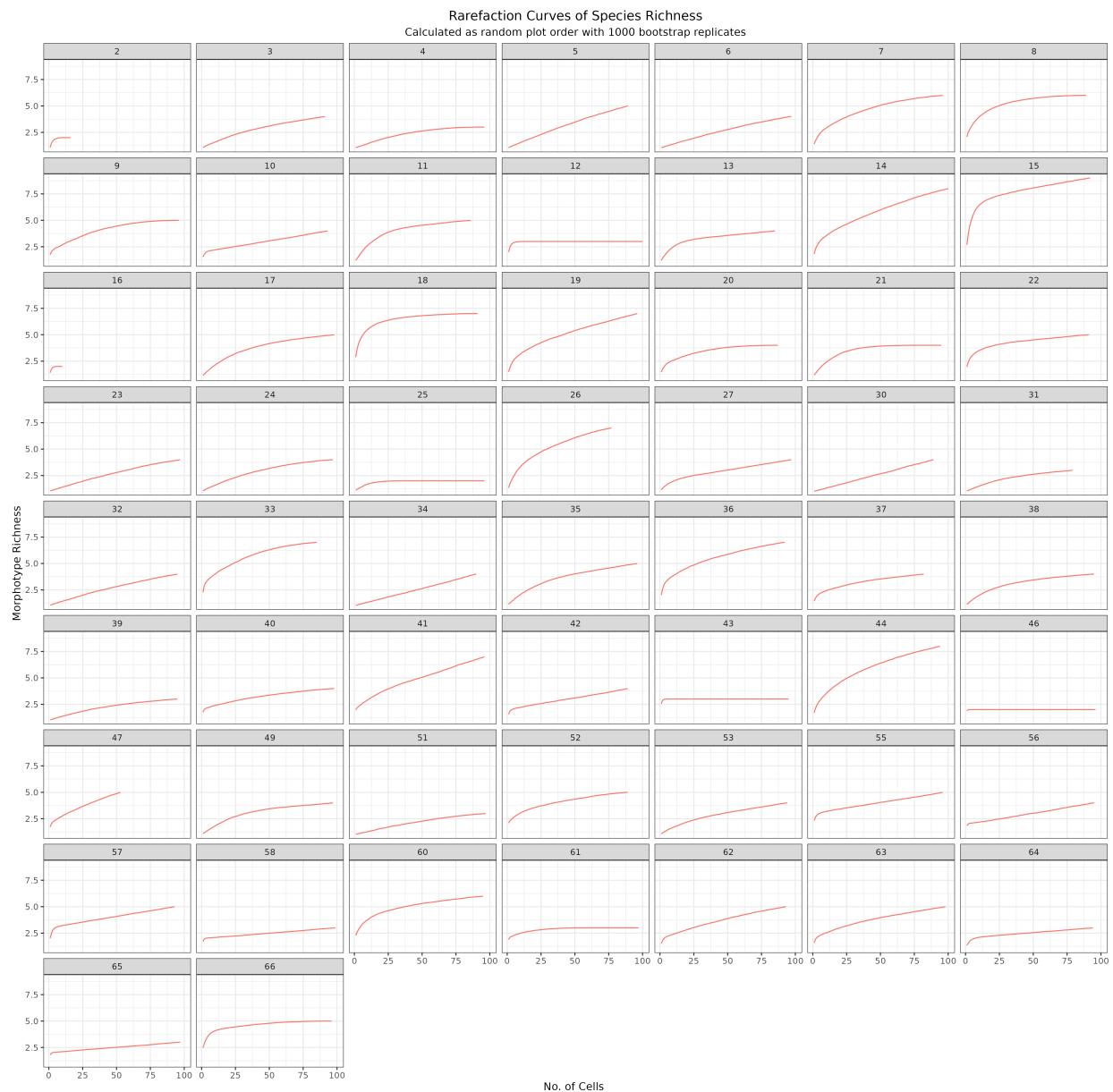
Percent matched reads per locus by sample



431 Appendix XX - Species Distribution Models Predictors

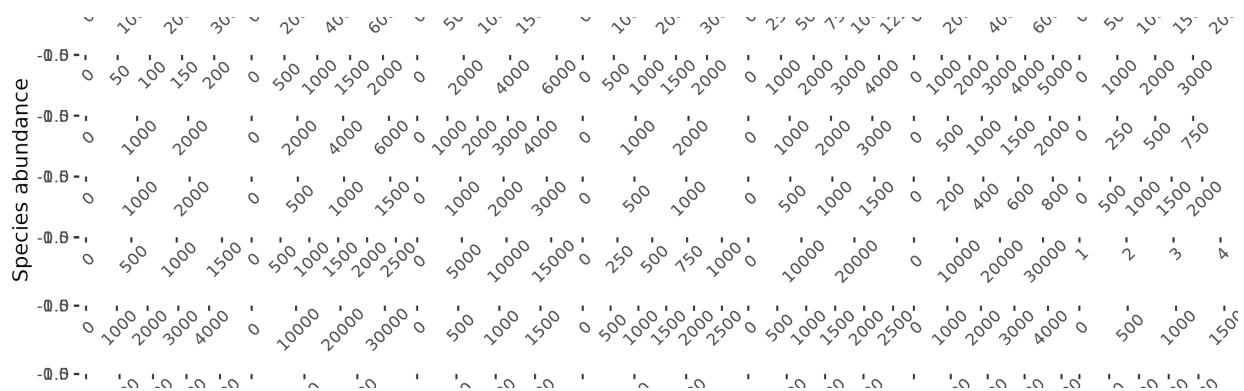
Layer	Description	Source
1.	Mean annual cloudiness - MODIS	Wilson et al. 2016
2.	Cloudiness seasonality 1 - MODIS	Wilson et al. 2016
3.	Cloudiness seasonality 2 - MODIS	Wilson et al. 2016
4.	Cloudiness seasonality 3 - MODIS	Wilson et al. 2016
5.	Beginning of the frost-free period	Wang et al.
6.	Climatic moisture deficit	Wang et al.
7.	Degree-days above 5C from	Wang et al.
8.	Mean annual precipitation	Wang et al.
9.	Mean annual precipitation as snow	Wang et al.
10.	Temperature seasonality	Wang et al.
11.	2015 Percent Grass/Herbaceous cover - MODIS	(MOD44B)
12.	2015 Percent Tree cover from Landsat 7/8	(GLCF)
13.	Soil probability of bedrock (R Horizon)	SoilGrids
14.	Soil organic carbon (Tonnes / ha)	SoilGrids
15.	Surface soil pH in H ₂ O	SoilGrids
16.	Surface soil percent sand	SoilGrids
17.	Soil USDA class	SoilGrids
18.	Topographic elevation	EarthEnv DEM
19.	Topographic elevation, moving window.	EarthEnv DEM
20.	Topographic percent slope	EarthEnv DEM
21.	Topographic wetness index	EarthEnv DEM
22.	Topographic aspect from	EarthEnv DEM
23.	Annual potential solar radiation computed	r.sun
24.	Estimated actual (w/-cloud) solar radiation r	.sun / Wilson et al. 2016
25.	Log-transformed distance to surface water Gl	obal Surface Water Explorer
26.	Percent surface water Gl	obal Surface Water Explorer

432 APPENDIX XX - Pollen Morphotype Richness Rarefaction Curves



433

434 Appendix XX - Pollen Morphotype Abundance Rarefaction Curves



435

Table 1: samples used in creating the Reference Library

Taxon	Family	Accession	Pres.	Locality	Date Col.	GenBank	Dist. (km)
<i>Cirsium parryi</i> (A. Gray) Petr.	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.IX.2020	tba	3.6
<i>Cirsium parryi</i> (A. Gray) Petr.	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.IX.2020	tba	3.6
<i>Ericameria parryi</i> (A. Gray) G.L. Nesom & Baird	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.IX.2020	tba	3.6
<i>Erigeron speciosus</i> (Lindley) De Cardolle	Asteraceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Erigeron subtrinervis</i> Rydb. Ex Porter & Britton	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.VII.2020	tba	3.6
<i>Helianthella quinquenervis</i> (Hook.) A. Gray	Asteraceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Helianthus multiflora</i> Nutt.	Asteraceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Heterotheca villosa</i> (Pursh) Shinners	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.IX.2020	tba	3.6
<i>Senecio sera</i> Hook.	Asteraceae	CHIC tba	P	Idaho, Idaho	26.VII.2020	tba	105.0
<i>Symplytrichum foliacium</i> (Lindl. Ex D.C.) G.L. Nesom	Asteraceae	CHIC tba	S	Illinois, McHenry	28.VII.2020	tba	1624.6
<i>Taraxacum officinale</i> F.H. Wigg.	Asteraceae	CHIC tba	S	Illinois, McHenry	28.VII.2020	tba	1624.6
<i>Mertenia ciliata</i> (James ex Torr.) G. Don	Boraginaceae	ID 1754185	S	Idaho, Valley	18.VI.2018	tba	979.3
<i>Mertenia ciliata</i> (James ex Torr.) G. Don	Boraginaceae	ID 169837	P	Idaho, Adams	10.VII.2014	tba	991.5
<i>Mertensia fusiformis</i> Greene	Boraginaceae	RMH 720522	P	Colorado, Gunnison	7.VI.1997	tba	44.8
<i>Campanula rotundifolia</i> L.	Campanulaceae	RMH 720600	P	Colorado, Gunnison	9.VII.1997	tba	38.9
<i>Lathyrus lanszwertii</i> Kellogg var. leucanthus (Ryd.) Dorn	Fabaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Lathyrus lanszwertii</i> Kellogg var. leucanthus (Ryd.) Dorn	Fabaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Lupinus argenteus</i> Pursh	Fabaceae	CHIC tba	P	Nevada, Pershing	29.V.2018	tba	971.2
<i>Lupinus argenteus</i> Pursh	Fabaceae	ISU 10387	P	Colorado, Gunnison	29.VI.2010	tba	0.2
<i>Lupinus bakeri</i> Greene	Fabaceae	ISU 10142	P	Colorado, Gunnison	15.VIII.2010	tba	2.6
<i>Vicia americana</i> Muhl. ex Willd.	Fabaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Vicia americana</i> Muhl. ex Willd. var. minor Hook.	Fabaceae	CHIC tba	S	Montana, Carbon	4.VII.2019	tba	10020.8
<i>Frasera speciosa</i> Douglas ex Griseb	Gentianaceae	RMH 721930	P	Colorado, Gunnison	20.VI.1997	tba	66.2
<i>Frasera speciosa</i> Douglas ex Griseb	Gentianaceae	RMH 719305	P	Colorado, Gunnison	7.VII.1997	tba	19.8
<i>Hydrophyllum capitatum</i> Douglas ex. Benth	Hydrophyllaceae	RMH tba	P	Colorado, Mesa	30.VI.2011	tba	64.6
<i>Hydrophyllum capitatum</i> Douglas ex. Benth	Hydrophyllaceae	RMH tba	P	Colorado, Delta	8.VI.2011	tba	65.3
<i>Hydrophyllum fendleri</i> (Gray) Heller	Hydrophyllaceae	ID 161100	P	Washington, Yakima	9.VI.2008	tba	1429.7
<i>Hydrophyllum fendleri</i> (Gray) Heller	Hydrophyllaceae	ID 164040	P	Idaho, Idaho	27.V.2009	tba	1014.4
<i>Agastache pallidiflora</i> (Heller) Rydberg	Lamiaceae	CHIC tba	S	Arizona, Coconino	17.VII.2020	tba	617.7
<i>Chamerion angustifolium</i> (L.) Holub	Lamiaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Delphinium barbeyi</i> (Huth) Huth	Ranunculaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Delphinium nuttallianum</i> Pritz.	Ranunculaceae	ID 166162	P	Idaho, Gem	15.VI.2011	tba	982.5
<i>Delphinium nuttallianum</i> Pritz.	Ranunculaceae	ID 179376	P	Idaho, Gooding	29.IV.2017	tba	733.7
<i>Potentilla fruticosa</i> Pursh	Rosaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Potentilla fruticosa</i> Pursh	Rosaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Potentilla hippiana</i> Lehman.	Rosaceae	CHIC tba	S	New Mexico, Catron	15.VIII.2020	tba	573.8

(Continued on Next Page)

Table 1: samples used in creating the Reference Library (*continued*)

Taxon	Family	Accession	Pres.	Locality	Date Col.	GenBank	Dist. (km)
Potentilla pulcherrima Lehman.	Rosaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6

^a Accession includes both Herbarium and Accession number^b Pres. refers to Preservation method. 'S' denotes silica-gel dried, 'P' denotes pressed^c All Localities are in the United States of America

Table 1: All species present in the Reference Sequence Databases
(Kraken and BLAST)

Order	Family	Taxon
Alismatales	Potamogetonaceae	<i>Potamogeton wrightii</i>
Apiales	Apiaceae	<i>Osmorhiza aristata</i>
Asparagales	Amaryllidaceae	<i>Allium stamineum</i>
	Asparagaceae	<i>Streptopus amplexifolius</i>
Asterales	Asteraceae	<i>Anaphalis margaritacea</i> <i>Antennaria carpatica</i> <i>Antennaria dioica</i> <i>Artemisia sibirica</i> <i>Brickellia dentata</i> <i>Chrysanthemus greenei</i> <i>Cirsium pannonicum</i> <i>Cirsium parryi</i> <i>Cirsium vulgare</i> <i>Crepis pygmaea</i> <i>Ericameria parryi</i> <i>Erigeron ecuadoriensis</i> <i>Erigeron grandiflorus</i> <i>Erigeron rosulatus</i> <i>Erigeron uniflorus</i> <i>Helianthella quinquenervis</i> <i>Heterotheca villosa</i> <i>Hieracium avilae</i> <i>Hieracium jubatum</i> <i>Hymenoxys hoopesii</i> <i>Leucanthemum graminifolium</i> <i>Microseris lindleyi</i> <i>Omalotheca supina</i> <i>Packera quercetorum</i> <i>Pseudognaphalium attenuatum</i> <i>Pseudognaphalium frigidum</i> <i>Pseudognaphalium lacteum</i> <i>Pseudognaphalium oxyphyllum</i> <i>Rudbeckia hirta</i> <i>Scabrethia scabra</i> <i>Senecio adenophyllus</i> <i>Senecio algens</i> <i>Senecio apolobambensis</i> <i>Senecio candollei</i> <i>Senecio chionogeton</i> <i>Senecio formosus</i> <i>Senecio funcii</i> <i>Senecio gilliesii</i> <i>Senecio humillimus</i> <i>Senecio nutans</i> <i>Senecio puchei</i> <i>Senecio rufescens</i> <i>Senecio spinosus</i> <i>Senecio tephrosioides</i>

(Continued on Next Page)

Table 1: All species present in the Reference Sequence Databases (Kraken and BLAST) (*continued*)

Order	Family	Taxon
Boraginales	Campanulaceae	<i>Solidago chilensis</i> <i>Stilpnolepis intricata</i> <i>Symphyotrichum foliaceum</i> <i>Taraxacum cucullatum</i> <i>Taraxacum officinale</i>
		<i>Tonestus lyallii</i> <i>Townsendia formosa</i>
		<i>Campanula argaea</i> <i>Campanula rotundifolia</i>
		<i>Cynoglossum amplifolium</i> <i>Cynoglossum anchusoides</i>
		<i>Cynoglossum pringlei</i> <i>Mertensia ciliata</i> <i>Mertensia fusiformis</i>
	Hydrophyllaceae	<i>Hydrophyllum canadense</i> <i>Hydrophyllum capitatum</i> <i>Hydrophyllum fendleri</i> <i>Nemophila menziesii</i>
		<i>Arenaria globiflora</i> <i>Arenaria serpyllifolia</i> <i>Cerastium arvense</i> <i>Cerastium lanceolatum</i>
		<i>Minuartia recurva</i> <i>Odontostemma leucasterium</i> <i>Pseudostellaria heterophylla</i> <i>Sagina procumbens</i>
		<i>Schizotechium monospermum</i> <i>Shivparvania glanduligera</i> <i>Stellaria graminea</i> <i>Stellaria holostea</i>
		<i>Stellaria obtusa</i> <i>Rumex induratus</i> <i>Rumex spinosus</i>
Celastrales	Celastraceae	<i>Parnassia faberi</i> <i>Parnassia palustris</i> <i>Paxistima canbyi</i> <i>Gaultheria prostrata</i>
		<i>Moneses uniflora</i> <i>Orthilia secunda</i>
		<i>Vaccinium vitis-idaea</i> <i>Collomia grandiflora</i> <i>Ipomopsis aggregata</i>
		<i>Phlox douglasii</i>
Fabales	Primulaceae	<i>Androsace studiosorum</i> <i>Androsace vitaliana</i>
		<i>Astragalus pelecinus</i>
		<i>Lupinus argenteus</i>
		<i>Lupinus sericeus</i>

(Continued on Next Page)

Table 1: All species present in the Reference Sequence Databases
(Kraken and BLAST) (*continued*)

Order	Family	Taxon
Gentianales	Gentianaceae	<i>Vicia americana</i> <i>Frasera speciosa</i> <i>Gentiana cruciata</i>
Hyphomicrobiales	Xanthobacteraceae	<i>Azorhizobium caulinodans</i>
Lamiales	Lamiaceae	<i>Agastache pallidiflora</i>
Liliales	Colchicaceae	<i>Prosartes smithii</i>
	Liliaceae	<i>Erythronium dens-canis</i>
	Melanthiaceae	<i>Anticlea elegans</i> <i>Veratrum viride</i>
Malpighiales	Hypericaceae	<i>Hypericum perforatum</i>
	Salicaceae	<i>Populus alba</i>
	Violaceae	<i>Viola odorata</i>
Myrtales	Onagraceae	<i>Chamaenerion angustifolium</i> <i>Epilobium canum</i> <i>Epilobium parviflorum</i>
Ranunculales	Berberidaceae	<i>Berberis sibirica</i>
	Papaveraceae	<i>Corydalis aitchisonii</i>
	Ranunculaceae	<i>Actaea heracleifolia</i> <i>Anemone anemonoides</i> <i>Anemone obtusiloba</i> <i>Aquilegia ecalcarata</i> <i>Caltha palustris</i> <i>Delphinium barbeyi</i> <i>Delphinium gracile</i> <i>Delphinium nuttallianum</i> <i>Pulsatilla chinensis</i> <i>Thalictrum thalictroides</i> <i>Thalictrum tuberosum</i> <i>Trollius europaeus</i>
Rosales	Elaeagnaceae	<i>Shepherdia argentea</i>
	Rosaceae	<i>Crataegus bipinnatifida</i> <i>Dasiphora fruticosa</i> <i>Geum ternatum</i> <i>Hedlundia austriaca</i> <i>Holodiscus argenteus</i> <i>Karpatiosorbus devoniensis</i> <i>Micromeles japonica</i> <i>Potentilla anserina</i> <i>Potentilla pulcherrima</i> <i>Potentilla tetrandra</i> <i>Rubus chingii</i>
Sapindales	Sapindaceae	<i>Acer campestre</i>
Saxifragales	Crassulaceae	<i>Rhodiola rosea</i> <i>Sedum nudum</i>
	Grossulariaceae	<i>Ribes rubrum</i>
	Saxifragaceae	<i>Lithophragma parviflorum</i> <i>Saxifraga biflora</i> <i>Saxifraga fortunei</i>

(Continued on Next Page)

Table 1: All species present in the Reference Sequence Databases
(Kraken and BLAST) (*continued*)

Order	Family	Taxon
		Saxifraga maderensis
		Saxifraga oppositifolia
		Saxifraga portosanctana
		Saxifraga x geum

x geum* \end{longtable}

448 Appendix XX - All Pollen Reference Slides Used to Establish Morphotypes

Table 1: All Pollen Voucher Slides Consulted

Taxon	Family	Locality	Accession	Type	Prepared by	Date
<i>Cymopterus lemnoides</i> (J.M. Coulter & Rose) Dorn	Apiaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Heracleum sphondylium</i> L.	Apiaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Ligusticum porteri</i> J.M. Coulter & Rose	Apiaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Osmorhiza depauperata</i> Phil.	Apiaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Maianthemum stellatum</i> (L.) Link	Asparagaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Achillea millefolium</i> L.	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Achillea millefolium</i> L.	Asteraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Acourtia wrightii</i> (A. Gray) Reveal & King	Asteraceae	NV, Clark, Gold Butte	tba	Novo	E.J.W.	2021
<i>Antennaria racemosissima</i> Hook.	Asteraceae	WY, Park, Ishawooa Mesa	tba	Novo	E.J.W.	2021
<i>Arnica latifolia</i> Bong.	Asteraceae	ID, Blaine, Wildhorse Canyon	tba	Novo	E.J.W.	2021
<i>Artemisia scopulorum</i> A. Gray	Asteraceae	CO, Hinsdale, Uncompahgre Peak	tba	Novo	E.J.W.	2021
<i>Canadanthus modestus</i> (Lindl.) G.L. Nesom	Asteraceae	ID, Idaho, Whiskey Creek	tba	Novo	E.J.W.	2021
<i>Chaenactis douglasii</i> (Hook.) Hook. & Arn.	Asteraceae	MT, Carbon, Pryor Mtn. Rd.	tba	Novo	E.J.W.	2021
<i>Erigeron corymbosus</i> Nutt.	Asteraceae	MT, Carbon, Pryor Mtn. Rd.	tba	Novo	E.J.W.	2021
<i>Erigeron flagellaris</i> A. Gray	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Erigeron speciosus</i> (Lind.) DC.	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Erigeron speciosus</i> (Lindl.) DC.	Asteraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Helianthella quinquenervis</i> (Hook.) A. Gray	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Heliotropium multiflorum</i> Nutt.	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Heliotropium multiflorum</i> Nutt.	Asteraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Heterotheca villosa</i> (Pursh) Shinners	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Heterotheca villosa</i> (Pursh) Shinners	Asteraceae	AZ, Coconino, Lake Mary Rd. & 209	tba	Novo	E.J.W.	2021
<i>Hymenoxys hoopesii</i> (A. Gray) Bierner	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Hymenoxys rusbyi</i> (A. Gray) Cockerell	Asteraceae	AZ, Coconino, Lake Mary Rd. & 209	tba	Novo	E.J.W.	2021
<i>Ionactis stenomeria</i> (A. Gray) Greene	Asteraceae	ID, Idaho, Marshall Mountains	tba	Novo	E.J.W.	2021
<i>Senecio hydrophilus</i> Nutt.	Asteraceae	ID, Custer, E. fl. Salmon River	tba	Novo	E.J.W.	2021
<i>Senecio integrerrimus</i> Nutt.	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Senecio serrula</i> Hook.	Asteraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Senecio wootonii</i> Greene	Asteraceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Solidago lepida</i> DC.	Asteraceae	ID, Idaho, American River	tba	Novo	E.J.W.	2021
<i>Sympotrichum foliacum</i> (Lindl. ex DC.) G.L. Nesom	Asteraceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Sympotrichum subspicatum</i> (Nees) G.L. Nesom	Asteraceae	ID, Custer, E. fl. Salmon River	tba	Novo	E.J.W.	2021
<i>Taraxacum officinale</i> F.H. Wigg	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Taraxacum officinale</i> F.H. Wigg	Asteraceae	IL, McHenry, Barrington	tba	Novo	E.J.W.	2021
<i>Lappula squarrosa</i> (Retz.) Dumort.	Boraginaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Mertensia ciliata</i> (James ex Torr.) G. Don	Boraginaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Mertensia fusiformis</i> Greene	Boraginaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Boechera</i>	Brassicaceae	NV, Washoe, Mt. Rose	tba	Novo	E.J.W.	2021
<i>Boechera stricta</i> (Graham) Al-Shehbaz	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Cardamine cordifolia</i> A. Gray	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Draba aurea</i> Vahl. Ex Hornem	Brassicaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014

(Continued on Next Page)

450 Appendix XX - All Pollen Reference Slides Used to Establish Morphotypes (con't)

Table 1: All Pollen Voucher Slides Consulted (*continued*)

Taxon	Family	Locality	Accession	Type	Prepared by	Date
<i>Draba spectabilis</i> Greene	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Erysimum capitatum</i> (Douglas ex Hook.) Greene	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Lepidium montanum</i> Nutt.	Brassicaceae	NM, Catron, Zuni Dry Lake	tba	Novo	E.J.W.	2021
<i>Smelowskia americana</i> Rydb.	Brassicaceae	ID, Blaine, Pioneer Mtns Crest	tba	Novo	E.J.W.	2021
<i>Thlaspi arvense</i> L.	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Campanula rotundifolia</i> L.	Campanulaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Campanula rotundifolia</i> L.	Campanulaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
Downingia	Campanulaceae	CA, Nevada, Truckee Meadows	tba	Novo	E.J.W.	2021
<i>Lonicera involucrata</i> (Richardson) Banks ex Spreng.	Caprifoliaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Minuartia nuttallii</i> (Pax.) Briq.	Caryophyllaceae	ID, Blaine, Wildhorse Canyon	tba	Novo	E.J.W.	2021
<i>Stellaria longifolia</i> Muhl. Ex Willd.	Caryophyllaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Parnassia kotzebuei</i> Cham. ex Spreng	Celastraceae	ID, Lemhi, Terrace Lakes	tba	Novo	E.J.W.	2021
<i>Sedum lanceolatum</i> Torr.	Crassulaceae	ID, Lemhi, Terrace Lakes	tba	Novo	E.J.W.	2021
<i>Astragalus robbinsii</i> (Oakes) A. Gray	Fabaceae	ID, Custer, Lake Creek	tba	Novo	E.J.W.	2021
<i>Glycyrrhiza lepidota</i> Nutt.	Fabaceae	ID, Butte, Warm Springs Creek	tba	Novo	E.J.W.	2021
<i>Lathyrus eucomis</i> Butters & H. St. John	Fabaceae	NM, Catron, Zuni Salt Lake	tba	Novo	E.J.W.	2021
<i>Lathyrus lanszwertii</i> var. <i>leucanthus</i> (Rydb.) Dorn	Fabaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Lathyrus lanszwertii</i> var. <i>leucanthus</i> (Rydb.) Dorn	Fabaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Lupinus argenteus</i> Pursh	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Lupinus argenteus</i> Pursh	Fabaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Lupinus crassus</i> Payson	Fabaceae	NV, Pershing, Star Peak Canyon	tba	Novo	E.J.W.	2021
<i>Lupinus sericeus</i> Pursh	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Melilotus albus</i> Medik.	Fabaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Trifolium hybridum</i> L.	Fabaceae	NM, Catron, Hwy 159	tba	Novo	E.J.W.	2021
<i>Trifolium pratense</i> L.	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Vicia americana</i> Muhl. Ex Willd.	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Vicia americana</i> Muhl. Ex Willd.	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Vicia americana</i> Muhl. Ex Willd.	Fabaceae	UT, Cache, Spawn Creek	tba	Novo	E.J.W.	2021
<i>Geranium</i>	Fabaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Geranium richardsonii</i> Fisch. Trautv.	Geraniaceae	NM, Catron, Jim Smith TH. Rd.	tba	Novo	E.J.W.	2021
<i>Phacelia</i> sp.	Geraniaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Iris missouriensis</i> Nutt.	Hydrophyllaceae	NV, Nye, Toiyabe Crest	tba	Novo	E.J.W.	2021
<i>Agastache palliflora</i> (A. Heller) Rydb.	Iridaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Erythronium grandiflorum</i> Pursh	Lamiaceae	AZ, Coconino, Lake Mary Rd. & 209	tba	Novo	E.J.W.	2021
<i>Linum lewisii</i> Pursh	Liliaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Zigadenus elegans</i> Pursh	Linaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Anticlea elegans</i> (A. Gray) Zomlefer & Judd	Melanthiaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Cistanthe/Calyptidium</i>	Melianthiaceae	ID, Blaine, Wildhorse Canyon	tba	Novo	E.J.W.	2021
<i>Chamerion angustifolium</i> (L.) Holub	Montiaceae	NV, Nye, Toiyabe Crest	tba	Novo	E.J.W.	2021
<i>Epilobium obcordatum</i> A. Gray	Onagraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Castilleja miniata</i> Douglas ex Hook.	Orobanchaceae	ID, Lemhi, Bighorn Crags	tba	Novo	E.J.W.	2021
		CO, Gunnison, Gothic	None	Image	H.M.B.	2010

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452 Appendix XX - All Pollen Reference Slides Used to Establish Morphotypes (con't)

Table 1: All Pollen Voucher Slides Consulted (*continued*)

Taxon	Family	Locality	Accession	Type	Prepared by	Date
<i>Castilleja sulphurea</i> Rydb.	Orobanchaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Pedicularis groenlandica</i> Retz.	Orobanchaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Pedicularis racemosa</i> Douglas ex Benth.	Orobanchaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Corydalis aurea</i> Willd.	Papaveraceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Erythranthe guttata</i> (DC.) G.L. Nesom	Phrymaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Penstemon wilcoxii</i> Rydb.	Plantaginaceae	MT, Missoula, Mission Mtns vic.	tba	Novo	E.J.W.	2021
<i>Collomia linearis</i> Nutt.	Polemoniaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Phlox condensata</i> (A. Gray) E.E. Nelson	Polemoniaceae	CO, Hinsdale, Uncompahgre Peak	tba	Novo	E.J.W.	2021
<i>Polemonium foliosissimum</i> A. Gray	Polemoniaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Polemonium occidentale</i> Greene	Polemoniaceae	ID, Custer, Lake Creek	tba	Novo	E.J.W.	2021
<i>Polemonium viscosum</i> Nutt.	Polemoniaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2010
<i>Bistorta bistortoides</i> (Pursh) Small	Polygonaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Eriogonum</i> spp.	Polygonaceae	NV, Washoe, Hwy 445	tba	Novo	E.J.W.	2021
<i>Polygala barbeyana</i> Chodat	Polygonaceae	NM, Eddy, Yeso Hills	tba	Novo	E.J.W.	2021
<i>Polygonum polygaloides</i> L.	Polygonaceae	MT, Missoula, Mission Mtns	tba	Novo	E.J.W.	2021
<i>Androsace filiformis</i> Retz.	Primulaceae	ID, Custer, Bradshaw Creek	tba	Novo	E.J.W.	2021
<i>Aquilegia coerulea</i> E. James	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Aquilegia coerulea</i> E. James	Ranunculaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Aquilegia elegantula</i> Greene	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Delphinium barbeyi</i> (Huth) Huth	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Delphinium nuttallianum</i> Pritz. Ex Walp.	Ranunculaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Ranunculus alismifolius</i> Geyer ex Benth.	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Ranunculus glaberrimus</i> Hook.	Ranunculaceae	ID, Lemhi, Agency Creek	tba	Novo	E.J.W.	2021
<i>Ranunculus inamoenus</i> Greene	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Ranunculus</i> spp.	Ranunculaceae	NV, Washoe, Mt. Rose	tba	Novo	E.J.W.	2021
<i>Thalictrum sparsiflorum</i> Tucz. Ex Fisch. & C.A. Mey.	Ranunculaceae	ID, Custer, E. flk. Salmon River	tba	Novo	E.J.W.	2021
<i>Dasiphora fruticosa</i> (L.) Rydb.	Rosaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Dasiphora fruticosa</i> (L.) Rydb.	Rosaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Fragaria virginiana</i> Duchesne	Rosaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Geum triflorum</i> Pursh	Rosaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Potentilla biennis</i> Greene	Rosaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Potentilla hippiana</i> Lehm.	Rosaceae	NM, Catron, Jim Smith TH.Rd.	tba	Novo	E.J.W.	2021
<i>Potentilla pulcherrima</i> Lehm.	Rosaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Potentilla pulcherrima</i> Lehm.	Rosaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Salix bebbiana</i> Sarg.	Salicaceae	ID, Custer, Lake Creek	tba	Novo	E.J.W.	2021
<i>Salix geyeriana</i> Andersson	Salicaceae	ID, Butte, Clyde	tba	Novo	E.J.W.	2021
<i>Mitella stauropetala</i> Piper	Saxifragaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Valeriana occidentalis</i> A. Heller	Valerianaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Viola canadensis</i> L.	Violaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010

* All Localities are in the United States of America

† Accession refers to whole-plant vouchers, all specimens are deposited at CHIC.

‡ Type Refers to whether both a physical and digital copy exist; 'Image' denotes only digital

§ Date refers to the Date of preparation.

- 1a: Pollen shed in clumps (tetrads/polyads); grains generally triangular, with an annulus subtending the porate apertures (go 34)
- 1b: Pollen generally dispersed as single units (monads); grains seldom if ever with annulus.
- 2a: Apertures porate, always lacking colpi
- 3a: grain outline from equatorial view circular
- 4a: Pores distributed along the equator.
- 5a: Pores > 5 (stephanoporate)
- 6a: Ornamentation homobrochate (~ *MENTZELIA*)
- 6b: Ornamentation otherwise (~ *POLYGALA*)
- 5b: Pores < 5 (*CURRENTLY OPEN*)
- 4b: Pores +/- distributed across grain (pantoporate)
- 7a: Ornamentation with striate ornamentation (~ *POLEMONIUM*)
- 7b: Ornamentation otherwise
- 8a: Ornamentation, slightly irregular - without regularly repeating features (scabrate) (~ *STELLARIA*)
- 8b: Ornamentation forming regularly repeating (reticulate) cells of varying shapes.
- 9a: spacing between the grid cells large (lophate), the walls of the cells with another set of projecting ornamentation (~ *OPUNTIA*)
- 9b: spacing between cells small, the wall of the cells without projecting features.
- 10a: Pores extending beyond the reticulate grids (~ *ARENARIA*)
- 10b: Pores extending beyond the reticulate grids (~ *PHLOX*)
- 3b: Outline from equatorial view otherwise (usually slightly triangular)
- 11a: Outline elliptic (*CURRENTLY EMPTY*)
- 11b: Outline not elliptic, grains often with acute, if rounded, angles along sides (e.g., triangular, polygonal) (*EMPTY*)
- 2b: Apertures with colpi, occasionally also with pores in addition (coporate)
- 12a: Grains with bristles tapering to points (echinate), and tri-colporate.
- 13a: Grains uniformly echinate, less the apertures. (Asteraceae 1)
- 13b: Grains with echinate bristles on ridges of lophae (Asteraceae 2)
- 12b: Grains without echinate ornamentation - this lead includes projections with ornamentation with round tips.
- 14a: Grains with either less than 3 apertures, or with two distinct ornamentation types (generally $\frac{1}{2}$ psilate, $\frac{1}{2}$ reticulate).
- 15a. Grains apparently lacking any apertures. (~ *IRIS*)
- 15b. Grains aperturate
- 16a. Ornamentation on one face of grain psilate, the other homobrochate (~ *ZIGADENUS + ANTICLEA*)
- 16b. Ornamentation psilate across both faces of grain (~*ERYTHONIUM*)
- 14b. Grains with either 3 or more apertures, or with an elongated spiral like aperture
- 17a. Grain with spiral like colpi
- 18a. Spiral with deep well-defined furrows (~ *ERYTHRANTHE GUTTATA*, syn. obsolete. *MIMULUS*)
- 18b. Spirals without well-defined grooves, ornamentation evidently perforate (~ *RANUNCULUS ALISMIFOLIUS*)
- 17b. Grains with colpi these not forming irregular spiral motifs.
- 19a. Grains elliptic, essentially perfectly cylindrical along longest axis, except for minor inundations along equatorial region. Apertures, of two types (heteroaperturate). (~ *BORAGINACEAE*)
- 19b Grains shaped similar or not, but never heteroaperturate.
- 20a. From a polar view, grains notably polygonal (hexagonal), also evident when seldom seen from a equatorial view. (~ *PHACELIA*/ maybe *Hydrophyllaceae*, *Hydrophyllum* not sampled)
- 20b. From a polar view, grains not with 6 convex apices

- 21a. Grains elliptic, with a short colporate aperture on each psilate face, the edges of each face and the apices with a distinct (homobrochate) textured ornamentation. (~ POLYGONUM)
- 21b. Grains otherwise, not featuring a mix of ornamentations independent of the apertures.
- 22a. Ornamentation perforate, the three colpi very short, their longest axis parallel to the equator rather than perpendicular. These colpi often times almost appearing to be slightly raised on an annulus like feature (~ LONICERA)
- 22b. Grains not as described in all aspects of the above.
- 23a. Apertures colporate
- 24a. Outline of grain in equatorial view circular, ornamentation smooth. (~MORPHOTYPE A).
- 24b. Grains otherwise
 - 25a Grains distinctly triangular from polar view (go 26)
 - 25b Grains elliptic (go 27)
 - 26a Grains very large, clearly strongly triangular in cross section. (~ GERANIUM)
 - 26b Grains smaller (SIZE), weakly triangular in cross section (~ POTENTILLA/DASIPHORA in part)
 - 27a Grains elliptic to weakly circular (~MORPHOTYPE B)
 - 27b Grains elliptic, much longer pole to pole than across equator.
 - 28a Grains with evident protrusions of the pore, colpi short, scarcely noticeable (~ APIACEAE)
- 23b. Apertures colpate
 - 30a Ornamentation psilate (~MORPHOTYPE C).
 - 30b Ornamentation otherwise
 - 31a Ornamentation homobrochate (~ MORPHOTYPE D)
 - 31b Ornamentation otherwise
 - 32a. Ornamentation bacculate, grains large, (~LINUM)
 - 32b. Ornamentation otherwise
 - 33a. Ornamentation of minor cross-corrugated grooves (fossulate) (~CORYDALIS)
 - 33b. Ornamentation of scarcely perceptible irregular features (scabrate) (~RANUNCULUS IN PART)

34a: Annula subtending the apertures – making grains appear more or less triangular; Pollen often with viscin threads (ONAGRACEAE)

34b: Apertures not annulate – grains appear more or less circular (~ERICACEAE)

Morphotype A: Trifolium, Lupinus, Glycrrhiza, Mitella, Geum

Morphotype B: Lupinus, Lathyrus, Potentilla, Androsace, Bistorta, Vicia

Morphotype C: Jeffersonia, Micranthes, Prunus, Delphinium, Androsace, Penstemon, Orthocarpus, Scutellaria, Aquilegia, Castilleja, Draba)

Morphotype D: Salix, Boechera

457 Appendix XX - Models used for Species Distribution Model Ensembles

458 *Generalised Linear Models (GLM)*

459 *Generalised Additive Models (GAM)*

460 Ensemble learning utilizes many sets of trees, each composed of many decisions, to create a single model.
461 Each independent variable (- or *feature*, may) become a node on the tree, a location on the tree where a
462 binary decision will move towards a predicted outcome. Each of the decision tree models which ensemble
463 learning utilizes is a weak models, each of which may suffer due to high variance or bias, but which produce
464 better outcomes than would be expected via random chance. When ensembled these models generate a strong
465 model, a model which should have more appropriately balanced variance and bias and predicts outcomes
466 which are more strongly correlated with the expected values than the individual weak models.

467 *Random Forest (RF)* the training data are continually bootstrap re-sampled, in combination with random
468 subsets of features, to create nodes which attempt to optimally predict a known outcome. A large number
469 of trees are then aggregated, via the most common predictions, to generate a final classification prediction
470 tree. Each individual prediction tree is generated independently of the others.

471 *Boosted Regression Tree (BRT)* An initial tree is grown, and all other trees are derived sequentially from it,
472 as each new tree is grown the errors in responses from the last tree are weighed more heavily so that the
473 model focuses on selecting dependent variables which refine predictions. All response data and predictor
474 variables are kept available to all trees.

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682 **List of Figures**

683 1	Phylogenetic tree of all biotically pollinated plant genera in the study area. The innermost	41
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Biotically pollinated plant genera with morphological or molecular data



Figure 1: Phylogenetic tree of all biotically pollinated plant genera in the study area. The innermost ring indicates every genus which Queen Bee's were observed to visit. The intermediate ring indicates that at least a single morphological pollen voucher slide was prepared for a member of the genus. The outermost ring indicates that sequence data were available for at least a member of that genus. Branch colors follow APG 4.

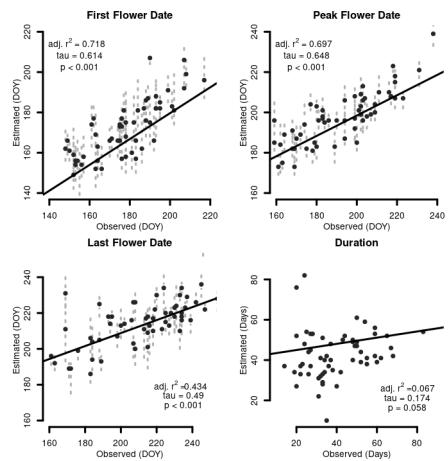


Figure 2: Modelled dates of when major flowering events occurred

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Table 2: Logistic regression assessing accuracy of SDMs

Metric	Value	Metric	Value
Accuracy (Training)	83.75	F-Score	0.84
Accuracy (Test)	84.00	AUC	0.92
Recall	81.03	Concordance	0.92
True Neg. Rate	86.97	Discordance	0.08
Precision	88.04	Tied	0.00

Table 3: Species Distribution Modeling evaluation contingency table

		Training		Testing	
		Absence	Presence	Absence	Presence
Absence	Absence	25620	3838	11130	1653
	Presence	6614	28248	2758	12024