1 Ecological drivers of ﬂower-leaf sequences: aridity and proxies for

2 pollinator attraction select for ﬂowering-ﬁrst in the American Plums

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17 **Summary**

18 • Across temperate forests many tree species produce ﬂowers before their leaves emerge. This ﬂower-

19 leaf phenological sequence, known as hysteranthy, is generally described as an adaptation for wind-

20 pollination. However, this explanation does not address why hysteranthy is also common in biotically-

21 pollinated taxa.

22 • We quantiﬁed ﬂower-leaf sequence variation in the American plums (*Prunus*, subspp. *Prunus* sect.

23 *Prunocerasus* ), a clade of insect-pollinated trees, using herbaria specimens and Bayesian hierarchical

24 modeling. We tested two common, but rarely interrogated hypotheses—that hysteranthy confers aridity

25 tolerance and/or pollinator visibility—by modeling the associations between hysteranthy and related

26 traits. To understand how these phenology-trait associations were sensitive to taxonomic scale and

27 ﬂower-leaf sequence classiﬁcation, we then extended these analyses to all *Prunus* species in North

28 America.

29 • Our ﬁndings across two taxonomic levels support the hypotheses that hysteranthy may help temporally

30 partition hydraulic demand to reduce water stress, and increase pollinator visibility—thereby reducing

31 selective pressure on inﬂorescence size.

32 • Our results provide foundational insights into the evolution of ﬂower-leaf sequences in the genus *Prunus*,

33 with implications for understanding these patterns in biotically-pollinated plants in general. Our ap-

34 proach suggests a path to advance these hypotheses to other clades, but teasing out drivers fully will

35 require new experiments.

36 Keywords: Deciduous forests, Flower-leaf sequences, Hysteranthy, Phenology, Plant hydraulics, Pollination,

37 Phylogeny

38 **Introduction**

39 Woody perennials are among a subset of plant types with the unique ability to seasonally begin reproduction

40 prior to vegetative growth. This ﬂowering-ﬁrst phenological sequence, known as hysteranthy, proteranthy or

41 precocious ﬂowering, is apparent in temperate deciduous forests around the globe (Rathcke & Lacey, 1985).

42 A number of studies suggest that this ﬂower-leaf sequence is under selection, and that hysteranthy can confer

43 performance advantages (Guo *et al.*, 2014; Gougherty & Gougherty, 2018; Buonaiuto *et al.*, 2021), but the

44 importance of variation in ﬂower-leaf sequences for maintaining ﬁtness may vary across functional types, taxa

45 and biomes.

46 The most common, and well-tested explanation for the evolution of hysteranthy in temperate forests is that it

47 is adaptive for wind-pollination, as leaﬂess canopies increase wind speeds for pollen transport and reduce the

48 likelihood of pollen interception by vegetation (Whitehead, 1969; Niklas, 1985). However, this explanation

49 does not address the widespread prevalence of hysteranthy in biotically-pollinated taxa found in temperate

50 regions. This number is not trivial; a recent analysis found that approximately 20% of the hysteranthy species

51 in Eastern Temperate Forests of North America are biotically-pollinated (Buonaiuto *et al.*, 2021).

52 Alternative hypotheses have been put forward to explain the advantage of hysteranthy in biotically-pollinated

53 species, but they have not been widely evaluated in the literature. Below, we brieﬂy review these hypotheses

54 and their predictions, then test their predictions using the American plums (*Prunus* subspp. *Prunus* sect.

55 *Prunocerasus* )—a widespread clade with high variability in ﬂower-leaf sequences—as a case-study. Our study

56 both clariﬁes the hypothesized function of ﬂower-leaf sequence variation in the genus *Prunus* and lays the

57 groundwork for understanding the origins of ﬂower-leaf sequence variation in biotically-pollinated taxa more

58 generally.

## 59 Hypotheses of hysteranthous ﬂowering in biotically-pollinated taxa

60 Water limitation hypothesis: In the dry-deciduous tropics of South and Central America, hysteranthy is

61 common (Rathcke & Lacey, 1985; Franklin, 2016), and is regarded as an important adaptation to alleviate

62 water stress by partitioning the hydraulic demand of ﬂowers and leaves across the season (Borchert, 1983;

63 Reich & Borchert, 1984; Franklin, 2016; Gougherty & Gougherty, 2018). Under this hypothesis, the function

64 of hysteranthous ﬂowering in temperate regions parallels that in the dry tropics. While temperate forests are

65 rarely water-limited in the early season during which ﬂowering and leaﬁng occur (Polgar & Primack, 2011),

66 there is still considerable variation in water availability in space and time within temperate regions of the

67 globe. With this hypothesis, we would expect to ﬁnd hysteranthous taxa in locations that are, on average,

68 drier than their non-hysteranthous relatives.

69 Insect visibility hypothesis: Hysteranthous ﬂowers are visually conspicuous in the landscape. Thus, as in

70 wind-pollinated taxa, hysteranthy in biotically-pollinated taxa may be an adaptation for pollination eﬃciency

71 as ﬂowering-ﬁrst species are easier for insect pollinators to locate (Janzen, 1967). A challenge to evaluating this

72 hypothesis is that correlated selection between ﬂower-leaf sequences and pollinator visibility could have either

73 a positive or negative relationship depending on the pollination environment. In one scenario, hysteranthy

74 may be associated with smaller ﬂoral displays: because ﬂowers are not obscured by leaves, they are easier

75 to see and there is weaker selection for increasing ﬂoral display size. In an alternative scenario, hysteranthy

76 could be associated with larger ﬂoral displays, especially in environments where plants are more often pollen-

77 limited and selection may favor both hysteranthy and increased ﬂoral display size to augment ﬂoral attraction

78 to visual pollinators.

79 In contrast to these functional hypotheses, hysteranthous ﬂowering could simply be a by-product of selection

80 for early ﬂowering. Species that ﬂower before their leaves inherently ﬂower early in the season. For example,

81 fruit development or dispersal constraints may drive early ﬂowering (Primack, 1987), and because spring

82 ﬂower phenology is less constrained by prior phenological events than leaf phenology (Ettinger *et al.*, 2018;

83 Savage, 2019), this selection for early ﬂowering could incidentally produce the hysteranthous phenological

84 sequence. Here, there is no speciﬁc adaptive advantage to hysteranthy; selection is not operating on the

85 relative timing of ﬂower and leaf emergence, but rather the absolute ﬂowering time alone. Rejection of the

86 above functional hypotheses might provide support to this null explanation.

87 A signiﬁcant challenge for robust testing of hysteranthy hypotheses is that most characterizations of ﬂower-

88 leaf phenological sequences are based on expert-opinion verbal descriptions (e.g. “ﬂowers before leaves” or

89 “ﬂower before/with leaves”), which make comparisons across taxa, time and space diﬃcult and sensitive

90 to observer bias (see Buonaiuto *et al.*, 2021). This problem can be overcome by adopting standardized

91 quantitative measures of plant phenology for observational studies and applying them to historical data

92 records. Herbarium records are an excellent source of data that can be leveraged for quantitative phenological

93 measurements (Willis *et al.*, 2017), but have not been widely used to investigate variability of ﬂower-leaf

94 sequences.

95 The American plums are a useful model clade to investigate drivers of hysteranthous ﬂowering in biotically-

96 pollinated species. The species that make up this group are distributed across the temperate zone of North

97 America and, like the genus *Prunus*, generally show pronounced inter-speciﬁc variation in ﬂower-leaf se-

98 quences. Usefully, species in this clade are well represented in herbaria records (Fig. 1a), making them a

99 tractable group to measure and assess variation in ﬂower-leaf sequences.

100 To interrogate the functional hypotheses for hysteranthous ﬂowering described above, we used herbaria records

101 to quantify variation in ﬂower-leaf sequences of the American plums. Then we combined environmental

102 attributes, biological traits and phylogenetic data in statistical models designed to evaluate whether the

103 observed associations between ﬂower-leaf sequences and morphological and environmental traits match the

104 predicted associations of the hysteranthy hypotheses. Finally, we compared our ﬁndings in this clade to

105 patterns observed in larger genus *Prunus* to test whether these phenology-trait associations were sensitive to

106 taxonomic scale and ﬂower-leaf sequence classiﬁcation.

# 107 Materials and Methods

## 108 Quantifying ﬂower-leaf sequence variation

109 We obtained digital herbarium specimens of the American plums from the Consortium of Midwest Herbaria

110 (CMH) Database (of Midwest Herbaria, 2023). Specimen collection dates ranged from 1844-2020, with the

111 majority collected between 1950-2000. To quantify ﬂower-leaf sequence variation in this group we randomly

112 sampled 200 specimens for each species and scored the phenological development of ﬂowers and leaves; we

113 used a modiﬁed BBCH scale for woody plants designed to evaluate vegetative and reproductive phenological

114 progress through a standardized quantitative index (Finn *et al.*, 2007). For species with less than 200

115 specimens in the collection, we included all available specimens. In total, we evaluated the phenology of 2521

116 specimens, but only specimens with visible ﬂowers were included in this analysis. We also removed specimens

117 with ﬂowering dates that were major outliers from the observed ﬂowering period of each species. We removed

118 outliers visually, and by excluding observations that were beyond three standard deviations of the median

119 ﬂowering time for each species (*n*=9). Our ﬁnal analyses included 1000 specimens (see Tab. S1 for number

120 of observations/species).

121 We reconstructed the phylogenetic relationships among species in this group based on the tree topology in

122 Shaw & Small (2004). We inferred branch lengths following the method of Grafen & Hamilton (1989) in

123 which node heights are estimated in proportion to number of subtending taxa using the R package “ape” (E.

124 Paradis and K. Schliep, 2019).

125 To quantify ﬂower-leaf sequence variation, we ﬁt an ordinal, hierarchical, Bayesian phylogenetic mixed model

126 (de Villemeruil P. & Nakagawa, 2014) designed to assess the likelihood an individual would be at any given

127 vegetative BBCH phase while ﬂowering. Our model predicted leaf stage (*yi*, ordinal, with six categories

128 representing stage from 1 for “buds closed” and 6 for “leaf expansion complete”) as a function of species and

129 additional phylogenetic eﬀects. Because hysteranthy co-varies with ﬂowering time (i.e., ﬂowering ﬁrst species

130 will generally ﬂower earlier than other species, on average), and collection dates were not evenly distributed

131 across the ﬂowering season (see Fig. S1), we included day of year of observation as an additional predictor.

132 Additionally, because climate change could aﬀect the interval between ﬂowering and leafout over the course

133 of our time series, we included the year of collection of each specimen as a covariate. Following previous

134 conventions for modeling the possible eﬀects of climate change on spring phenology, we parameterized *year*

135 as a hinge variable, using 1980 as a break point (Stocker *et al.*, 2013; Buonaiuto *et al.*, 2021).

136 The model is written below:

137

 2 *if zi ∈* (0*, c*2)

 1 *if zi <* 0

*y* =  3 *if zi ∈* (*c*2*, c*3)

*i*

4 *if zi ∈* (*c*3*, c*4)

 5 *if zi ∈* (*c*4*, c*5)

 6 *if zi > c*5

138

139

*zi* = *α* + *αphylo* + *αsp* + *β*day of year[sp] *∗ X*day of year + *βyear ∗ Xyear* + *ϵi*

140 *ϵi ∼ logistic*(0*,* 1)

141

142 where *yi* is the ordinal outcome (leaf stage; as 1,2,...6 categories). *c*2*...*5 are the estimated cutpoints between

143 leaf stages on the logit scale and *Xyear* is the year the specimen was collected *−* 1980. *zi* is the linear

144 component of the underlying latent variable model.

145 *α* describes an intercept for each category [1,2,...6] and slopes (*β*day of year and *βyear*) are constant across

146 cutpoints. *β*day of year also varies among *species* while *βyear* is a pooled estimate across species.

147 The inﬂuence of the phylogeny (*αphylo*) was modeled as:

148

*αphylo ∼* normal(0*, COR*[*σ*2 ])

*phylo*

149 The *α* for species eﬀects independent of the phylogeny was modeled as:

150

*αsp ∼* normal(0*, σ*2 )

*species*

151 We used our model to predict the probability that each species would be observed at a given vegetative

152 BBCH stage during ﬂowering for each day of the ﬂowering period of each species by extracting 1000 ran-

153 dom draws from the posterior distribution. Next, for each day of the ﬂowering season, we summed the

154 predicted likelihood that species would be at BBCH 0 (“bud closed”), BBCH 07/09 (“bud break”) or BBCH

155 11 (“start of leaf unfolding) vs. BBCH 15 (“leaf unfolding”), BBCH 17(“most leaves unfolded”), BBCH 19

156 (“leaf expansion complete”)—this allowed us to quantify the likelihood that a species would be hysteranthous

157 or non-hysteranthy respectively for each day of the season. We chose the BBCH 11/BBCH 15 boundary to

158 deﬁne hysteranthous ﬂowering because this is the earliest point in development when most leaves are unfurled

159 enough to visually obscure ﬂowers and transpire. Finally, we used these estimates to develop a ﬂower-leaf

160 sequence index: for this, we summed the likelihood of hysteranthy vs. non-hysteranthy across the full ﬂower-

161 ing period of each species, with 0 being never hysteranthous and 1 being always hysteranthous. To evaluate

162 the sensitivity of our model to choice of cutoﬀ, we also calculated a hysteranthy index using an alternative

163 cutoﬀ at the BBCH 09/BBCH 11, which did not alter the species’ ranks on the index (see Tab. S2).

164 To better understand how within-season dynamics aﬀected our inference, we also reﬁt our model excluding

165 *day of year* as a predictor. This version of the model did not substantially alter the species’ ranks on the index

166 or our inference about the relationships between ﬂower-leaf sequence variation and the trait representing the

167 main hysteranthy hypotheses (Tab. S2, Tab. S3).

## 168 Evaluating hysteranthy hypotheses

169 To test the hypotheses of hysteranthy, we ﬁrst recorded petal length measurements directly from herbarium

170 specimens. For these morphological measurements, we sampled 321 specimens and measured the petal length

171 of up to 10 randomly selected petals per specimen (*n*=2757) using ImageJ image processing software (see

172 Tab. S2, for *n* per species).

173 To assess aridity tolerance, we computed the average Palmer Modiﬁed Drought Index score from June-August

174 (hereafter: PDSI), obtained from Gille *et al.* (2017) for every *Prunocerasus* specimen in the database (*n*=2305,

175 see Tab. S2, for *n* per species). PDSI is a unitless, standardized drought index that integrates temperature

176 and precipitation data to estimate relative dryness in time and space (Heim, 2002). Negative PDSI values

177 indicate more arid conditions and positive values wetter conditions. For any specimens that lacked accurate

178 geo-location information, we extracted PDSI values at the county centroid of the herbaria specimen.

179 Because all of our measurements were made on diﬀerent individuals—with diﬀerent sample sizes—we used

180 two diﬀerent modeling approaches to test the relationship between ﬂower-leaf sequence index scores, aridity

181 tolerance and ﬂoral displays.

182 First we computed species-level means of PDSI and petal length and used a beta regression to evaluate the

183 relationship between ﬂower-leaf sequences, PDSI, petal length and their interaction. We standardized the

184 units of all predictors through *z*-scoring (Gellman & Hill, 2007) to make their eﬀect size estimates directly

185 comparable within the following model structure:

186

187 *yi* = (*µ, µ*(1 *− µ*)*/*(1 + *ϕ*)

188

189 where *µ* and *ϕ* are the two shape parameters of the beta regression. Due to the limited sample size of this

190 analysis (13 species), we only modeled the eﬀect of our predictors on the mean parameter, *µ*, and ﬁt a grand

191 intercept for the precision parameter, *ϕ*.We modeled the *µ* parameter as:

192

193

194

*µ* = *α* + *βPDSI ∗ XPDSI* + *β*petal length *∗ X*petal length + *βPDSIx*petal length *∗* (*XPDSI* )(*X*petal length)

195 We chose this model structure because it allowed us to assess the additive and interactive eﬀects of PDSI

196 and petal size on ﬂower-leaf sequences. However, by using mean trait values as predictors, we could not

197 incorporate within-species variation in these trait/environmental predictors or account for their phylogenetic

198 structure. To understand how these factors aﬀected our inferences about the relationship between ﬂower-leaf

199 sequences and traits, we ﬁt two additional models to estimate relationship between ﬂower-leaf sequences index

200 values and PDSI, and between ﬂower-leaf sequences index values and petal size separately which included

201 the intra-speciﬁc variation and phylogenetic structure of each of these traits (see Supporting Information:

202 Extended Methods for details). This alternative modeling approach produced similar results for phenology-

203 trait relationships to our main model.

204 **Hysteranthy in the larger genus *Prunus***

205 To better understand how the patterns we identiﬁed in the American Plums clade scaled across a coarser

206 taxonomic resolution, we also evaluated the relationship between hysteranthous ﬂowering and hypothesis-

207 related traits for additional *Prunus* species native to, or established in, North America (*n*=32). For this

208 analysis, we obtained categorical descriptions of ﬂower-leaf sequences and mean estimates of the number of

209 ﬂowers per inﬂorescence as a proxy for ﬂoral investment from the Flora of North America (Rohrer, 1993+).

210 We extracted PDSI values for all herbaria observations of those species in the Consortium of Midwest Herbaria

211 database (*n*=23,272) as described above.

212 To account for the inﬂuence of evolutionary relationships among species, we reconstructed the phylogenetic

213 relationships in the genus based on the tree topology in Chin *et al.* (2014). As above, we computed branch

214 lengths with the R package “ape” (E. Paradis and K. Schliep, 2019).

215

216

As above, we standardized the units of all predictors through *z*-scoring (Gellman & Hill, 2007). The model structure is:

 1 *if zi <* 0

*y* =  2 *if zi ∈* (0*, c*2)

*i*

3 *if zi ∈* (*c*2*, c*3)

 4 *if zi > c*3

217 *zi* = *α*+*αphylo*+*βPDSI∗XPDSI* +*β*ﬂoral investment*∗X*ﬂowers/inﬂorescence+*βPDSIx*ﬂoral investment(*XPDSI* )(*X*ﬂowers/inﬂorescence)+

218 *ϵi*

219

*ϵi ∼ logistic*(0*,* 1)

220 where *yi* is the ordinal outcome of ﬂower-leaf sequence category ( “ﬂowers after leaves”=1, “ﬂowers with

221 leaves”=2, “ﬂowers before/with leaves”=3 and “ﬂowers before leaves”=4) and *c*2*...*3 are the estimated cutpoints

222 between categories on the logit scale. *zi* is the linear component of the underlying latent variable model. *α*

223 describes a grand intercept, and we modeled the inﬂuence of phylogeny (*αphylo*) as above. Note that this

224 model includes four ordinal categories, while our model of the American Plums clade included six, due to the

225 diﬀerent underlying structures of the two datasets.

226 **Model runs**

227 We ﬁt all models in the R package “brms” (Bu¨rkner, 2018) using weakly informative priors, and four chains.

228 For the models aimed at“Quantifying ﬂower-leaf sequence variation” and“Evaluating hysteranthy hypotheses”

229 in the American plums, we ran the models with a warm-up of 3000 iterations, and 4000, and 5000 sampling

230 iterations respectively, for a total of 4000 and 8000 sampling iterations across all chains. For the “Hysteranthy

231 in the larger genus *Prunus*” model, we used a warm up of 6,000 iterations and 8,000 sampling iterations for

232 a total of 8000 sampling iterations to maximize the eﬀective sampling size. Model ﬁts were assessed with *R*ˆ

233 *<*1.01, high eﬀective sample sizes, and no divergent transitions. We provide mean estimates with uncertainty

234 intervals in-text, and 50% and 89% intervals for all ﬁgures and tables.

235 **Results**

## 236 Quantifying ﬂower leaf sequences in the American plums

237 We found substantial inter-speciﬁc diﬀerences in ﬂower-leaf sequences within the American plums (Fig. 1b,

238 Fig. 2a). Several species (*P. mexicana*, *P. umbellata*, *P. angustifolia*, *P. maritima* and *P. gracilis*) were

239 most likely to be hysteranthous for all—or most—of their ﬂower period, while for others, (*P. americana*, *P.*

240 *munsoniana*, *P. alleghaniensis*, *P. nigra*, *P. hortulana*, *P. texana* and *P. rivularis* ), hysteranthous ﬂowering

241 was only likely in the early part of their ﬂowering session. One species, *P. subcordata*, was unlikely to be

242 hysteranthous at any point in its ﬂowering period (Fig. 2a). These relative ranking of species’ hysteranthy

243 likelihoods were consistent with our alternative method for constructing the hysteranthy index (Tab. S2, Fig.

244 S2).

245 Across all species of American Plums, day of year increased the likelihood of ﬂowering during a later vegetative

246 phenological stage (Fig. 2b). Year of observation did not substantially impact the likelihood of hysteranthy

247 for this taxonomic group (Fig. 2b).

## 248 Associations between hysteranthy and environmental and morphological traits

249 In the American plums, predominately hysteranthous species had marginally smaller ﬂowers and occurred in

250 historically drier localities than species with more overlap between ﬂowers and leaves (i.e., increased likelihood

251 of hysteranthy was negatively associated with PDSI and petal length without a substantial interaction between

252 them, Fig. 3a; parameter estimates from this model were *βPDSI* : *−*0*.*47*, UI*89[*−*0*.*96*,* 0*.*01], *β*petal length :

253 *−*0*.*14*, UI*89[*−*0*.*54*,* 0*.*24] *βPDSIx*petal length : *−*0*.*14*, UI*89[*−*0*.*91*,* 0*.*65]). These estimates were comparable to

254 estimates from models where we treated each predictor separately and accounted for phylogeny (Fig. S3), and

255 where we used the hysteranthy index derived from models that did not include day of year as a predictor (Tab.

256 S3). The direction and magnitude of the estimated eﬀects support the predictions of the water-limitation

257 hypothesis and marginally support the predictions of the insect-visibility hypothesis.

258 In the larger genus *Prunus*, hysteranthous species had smaller inﬂorescences and were found in drier locations

259 (Fig. 4a, b; i.e., there was a negative association between hysteranthy and PDSI and number of ﬂowers

260 per inﬂorescence, as well as a substantial negative interaction between them, parameter estimates from

261 this model were *βPDSI* : *−*8*.*0*, UI*89[*−*16*.*6*, −*2*.*44], *βflowers/inflorescence* : *−*15*.*5*, UI*89[*−*31*.*46*, −*5*.*56] and

262 *βPDSIxflowers/inflorescence* : *−*13*.*06*, UI*89[*−*28*.*53*, −*2*.*93]). The direction and magnitude of the estimated

263 eﬀects support the predictions of both the water-limitation hypothesis and the insect-visibility hypothesis.

264 The estimated eﬀects of ﬂoral traits and their interactions with PDSI were stronger in the larger genus *Prunus*

265 than in the American plums clade. This is not surprising given that all species in the American plums clade

266 have solitary ﬂowers, making the variation in ﬂoral display size highly constrained. By contrast, *Prunus*

267 species included in our secondary analysis include those with solitary ﬂowers and species with as many as 100

268 ﬂowers per inﬂorescence—substantially more variation in both ﬂoral investment and in hydraulic demand.

269 This suggests that the correlated selection between ﬂower-leaf sequences and these ﬂoral traits may be more

270 pronounced at coarser taxonomic resolutions, where underlying trait variation is greater.

271 **Discussion**

272 Using North American *Prunus* species as a case study, our results indicate that ﬂower-leaf sequences are likely

273 under selection. We show that variation in ﬂower-leaf sequences across species may reﬂect adaptive tradeoﬀs

274 between a) the timing of investment in reproduction relative to the timing of resumption of carbon acquisition

275 through leafout, and b) other aspects of plant performance, such as environmental tolerance and pollinator

276 attraction strategies that we investigated in this study. We show that hysteranthous ﬂowering is associated

277 with historic aridity (PDSI) and smaller ﬂower displays in both the American plums, and more broadly

278 across *Prunus* species native to, or established in, North America. The relationships between hysteranthy

279 and aridity, and hysteranthy and ﬂoral display size support the predictions of the water limitation hypothesis

280 and the insect visibility hypothesis.

281 Our models estimated a strong relationship between aridity (PDSI) and ﬂower-leaf sequences at both taxo-

282 nomic scales we studied, but the relationship between ﬂoral display size and ﬂower-leaf sequences predicted

283 by the insect visibility was better supported at the coarser taxonomic scale of the full genus *Prunus* than

284 in the American plums clade. While species in the American plum clade have solitary ﬂowers, they are

285 still clustered on branches, so it is unlikely the unit of attraction that pollinators are responding to is the

286 individual ﬂower, which may explain why the relationships between hysteranthy and petal size we observed

287 were weak (Fig. 3b). The inﬂorescences of the larger genus *Prunus*, are probably a better proxy for diﬀer-

288 ences in units of attraction, which may explain their stronger association with ﬂower-leaf sequences variation

289 among species (Fig 4). This contrast may suggest that associated selection between ﬂower-leaf sequences and

290 pollinator traits has more strongly inﬂuenced inﬂorescence architecture than the morphology of individual

291 ﬂowers though our estimates at both scales agreed in directionality (i.e., hysteranthy associated with smaller

292 ﬂoral displays).

293 Under the insect visibility hypothesis, ﬂoral display size could either be positively or negatively associated

294 with hysteranthy depending on the pollination environment. The association between hysteranthy and smaller

295 ﬂower displays that we found supports the prediction that increased visibility of hysteranthous ﬂowers reduces

296 selection pressure on ﬂower display size. These results ﬁt with other comparative anatomy studies in plants

297 that report hysteranthous species typically have smaller inﬂorescences than non-hysteranthous relatives (Gu-

298 natilleke & Gunatilleke, 1984), and studies on pollinator foraging behavior that suggest the presence of leaves

299 substantially alters the visual perception of pollinators (Rivest *et al.*, 2017; Forrest & Thomson, 2009).

300 Our support for both the water limitation hypothesis and insect visibility hypothesis (especially in the larger

301 genus *Prunus*), and the strong positive interactions between PDSI and ﬂoral investment that we observed in

302 the larger genus *Prunus*, highlight that these hypotheses are not mutually exclusive, and could be related.

303 Selection on ﬂoral size represents a classic evolutionary tradeoﬀ where larger ﬂoral displays may generally

304 be more eﬀective for attracting pollinators but demand more resources, including water, to maintain turgor

305 and reproductive function than smaller ones (Galen *et al.*, 1999; Lambrecht & Dawson, 2007). With this

306 trade-oﬀ, reproductive displays are often small in harsher environments (Lambrecht, 2013; Teixido *et al.*,

307 2016), and hysteranthy could represent a compensatory mechanism that both reduces hydraulic demand

308 while increasing pollination eﬃciency in these environments. Studies that have compared the transpiration

309 rates among ﬂowers and leaves provide insights to the potential importance of hysteranthy for maintaining

310 water status. Measurements of water movement (transpiration rates, sap ﬂow, hydraulic conductivity) to

311 ﬂowers range from 20%-60% of that of leaves under comparable conditions (Whiley *et al.*, 1988; Roddy &

312 Dawson, 2012; Liu *et al.*, 2017; McMann *et al.*, 2022). This level of additional hydraulic demand can drive

313 loss of stomatal conductance and decrease photosynthetic rates (Galen *et al.*, 1999).

314 The PDSI records that we incorporated in this study—spanning over two millennia—oﬀer additional insights

315 into why the water limitation hypothesis may still be relevant to temperate forest regions that are typically

316 well saturated in the spring. These records reveal that the southeastern United States (the region in which

317 much of our data originates) was once much drier than it has been in contemporary times (Cook *et al.*, 2010,

318 Fig S4), suggesting that the spatial patterns of hysteranthy we see in the American plums may represent a

319 lagged-correlation with historical climate conditions.

320 Despite this evidence that hysteranthy can reduce hydraulic demand in dry environments, hysteranthous

321 species in the American plum clade are not found—even historically—in extremely arid locations (PDSI

322 values typically range from -4 to 4, although the values that we observed in our analyses were more restricted,

323 ranging from -0.5 to 0.2). This contrasts with hysteranthous species in the dry tropics where this phenological

324 pattern appears to allow them to tolerate more extreme aridity (Franklin, 2016). But the ﬂower-leaf sequences

325 of the hysteranthous species in our study were markedly diﬀerent from patterns of hysteranthy in these dry-

326 tropics where the water limitation hypothesis was initially proposed. While ﬂowering can precede leafout

327 by as much several weeks for species in the American plums, the process of fruit development, which is also

328 water intensive, occurs when leaves are present. By contrast, in the dry tropics hysteranthous ﬂowering is

329 initiated at the time of leaf drop (Borchert, 1983; Franklin, 2016); thus, the full reproductive cycle occurs in

330 the leaﬂess period. The comparatively small window of leaﬂess reproductive development in our temperate

331 clade suggests that hysteranthy may allow temperate species to occupy marginally drier environments than

332 non-hysteranthous species, but may not facilitate species’ persistence under extreme aridity.

## 333 Inter-and intra-speciﬁc variation in ﬂower-leaf sequences

334 We developed a novel approach to assessing ﬂower-leaf sequences that scales from quantitative, individual-

335 level observations to species-level characterizations. With this approach, we were able to—for the ﬁrst

336 time—quantitatively assess intermediate cases of hysteranthy (such as those that are typically described as

337 “ﬂowers before/with leaves”). Previous studies of hysteranthous ﬂowering have either excluded these cases

338 from their analyses (e.g.; Gougherty & Gougherty, 2018) or binned them with the well deﬁned cases (e.g.;

339 Buonaiuto *et al.*, 2021). We found that many American plum species expressed this intermediate ﬂower-leaf

340 sequence. Further, while our classiﬁcations broadly matched previous species-level analyses in this group by

341 Shaw & Small (2004), our approach identiﬁed substantial diﬀerences in ﬂower-leaf sequences among these

342 intermediate cases (Fig. 1b), which allowed us to assess the trait associations with this phenotype.

343 Our quantitative analysis of the American plums clade revealed that ﬂower-leaf sequences—often described

344 as a species-level trait—are highly variable within species (Fig. 2a). For almost all members of the clade,

345 hysteranthy was strongly predicted by the day of the observation (“day of year” in our model, Fig. 2b). In

346 many cases there was a high likelihood that individuals of a species may be observed at diﬀerent vegetative

347 stages during ﬂowering (Fig. 2a, Fig. S2). The variation we observed here could either suggest high levels of

348 local adaptation in ﬂower-leaf sequences or, alternatively, high levels of plasticity through which ﬂower-leaf

349 sequences respond to interannual variation in environmental conditions. Because our study was based on

350 herbaria records collected on diﬀerent individuals across space and time without repeat sampling, we could

351 not robustly estimate how much ﬂower-leaf sequences vary within vs. among species. However, this would be

352 an important next step for understanding how the environment and species interactions have shaped these

353 phenological patterns.

354 Interestingly, while there is substantial evidence that both ﬂowering and leaf phenology have advanced over

355 the last several decades in response to anthropogenic climate change (Menzel *et al.*, 2006; Cleland *et al.*,

356 2007; Augspurger & Zaya, 2020), we did not observe changes in ﬂower-leaf sequences over that time scale

357 in our dataset (Fig. 2b). This supports a recent ﬁnding that despite changes in both ﬂowering and leafout,

358 the time interval between them has remained relatively stable (Guo *et al.*, 2023), but does not preclude that

359 possibility that these the sequences will eventually be disrupted as climate change continues to become more

360 extreme in the future (Buonaiuto & Wolkovich, 2021).

361 **Future directions**

362 We focused on a well-studied, and economically important clade of morphologically similar species. Our

363 case-study provides a road map for evaluating the role of hysteranthy more generally in temperate biotically-

364 pollinated plant taxa (other groups with high interspeciﬁc ﬂower-leaf sequence variation include *Magnolia*,

365 *Rhododendron*, *Acer* and *Cornus*), and more broadly across taxa and biomes.

366 Combining the observational approach with novel experiments could further advance our collective under-

367 standing of the adaptive signiﬁcance of ﬂower-leaf sequences. To test the water-limitation hypothesis, re-

368 searchers could plant sister-taxa with contrasting ﬂower-leaf sequences in common environments across a

369 gradient of aridity, and evaluate their performance. To test the insect visibility hypothesis, researchers

370 should consider hysteranthy—and phenology in general—in the broader framework of tradeoﬀs in pollination

371 biology. The tradeoﬀ between phenology and pollination investment could not only consider ﬂower size, but

372 also the number of ﬂowers, nectar and pollen reward investment, volatiles between related hysteranthous and

373 non-hysteranthous taxa. Findings that hysteranthous species invest fewer resources into these other pollinator

374 attraction traits than non-hysteranthous relatives would support the insect visibility hypothesis. For a simple

375 experiment to test the pollinator visibility hypothesis, researchers could force hysteranthy/non-hysteranthy

376 phenotypes for the same genotype using environmental cues and systematically release pollinators to ob-

377 serve their preferences, search times and foraging behavior. If pollinators are more readily drawn to the

378 hysteranthous individuals, it would support hysteranthy as an adaptive trait for pollinator attraction.

379 With a better mechanistic understanding of the relationship between ﬂower-leaf sequences and ecological

380 performance, researchers could then use experiments to assess how diﬀerences in ﬂoral and leaf physiological

381 responses to temperature variation shape ﬂower-leaf sequences. The measurement and modeling approaches

382 we developed in our observational study can be readily implemented to analyze data from such experimental

383 settings, presenting an important opportunity to unite observations of broad ecological patterns with targeted

384 experimental manipulations to better understand both the evolutionary past and ecological future of ﬂower-

385 leaf sequences.

# 386 Competing Interests:

387 The authors declare no conﬂict of interest.

# 388 Author contributions

389 DMB, and EMW conceived of the manuscript; DMB and SC collected the data; DMB led the statistical

390 analyses with TJD and EMW; DMB led the writing of the manuscript. All authors contributed to writing

391 and gave approval for the submission.

392 **Data Availability**

393 The phenology and trait data collected for this study will be made available and archived at KNB: The

394 Knowledge Network for Biocomplexity (https://knb.ecoinformatics.org/) at the time of publication.

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492 **Figures**

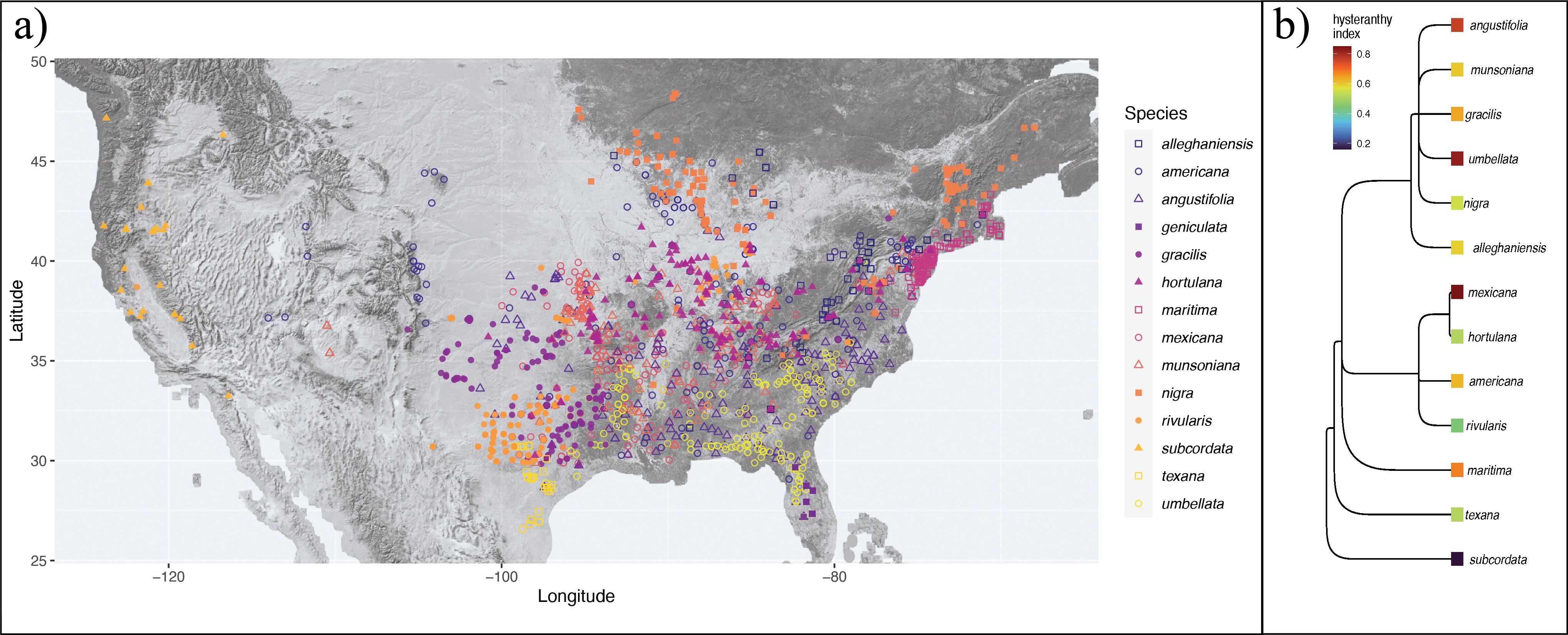


Figure 1: Geographic distribution and taxonomic relationships among the American plums. a) Maps the local- ities of all the herbaria records used in this study. b) Depicts phylogenetic relationships among the American plums and the likelihood they each species is hysteranthous across its full ﬂowering period, represented by a hysteranthy index where 0 is never hysteranthous and 1 is always hysteranthous. These designations are based on ordinal phylogenetic mixed models. Tree topology is from Shaw & Small (2004)

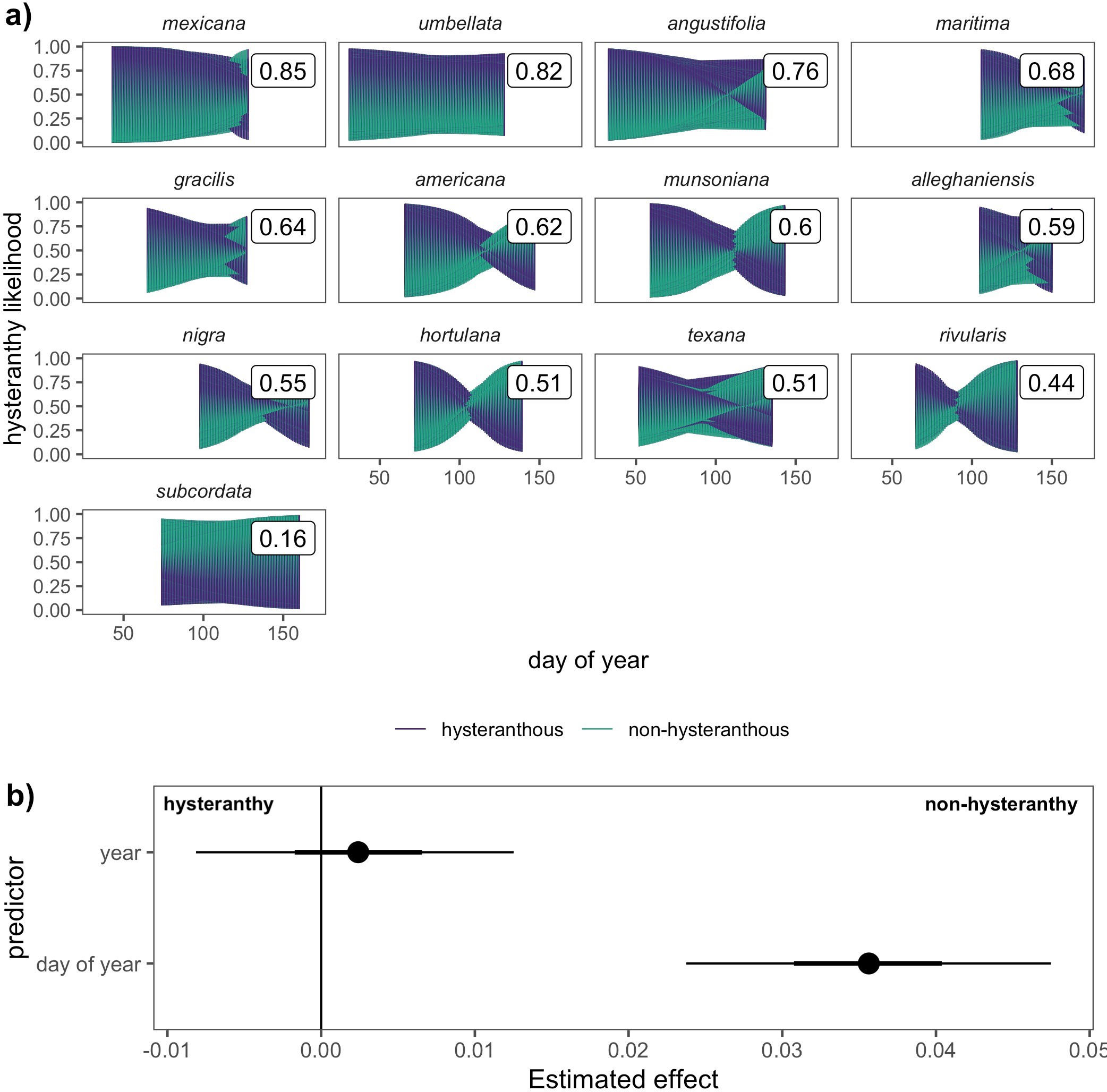


Figure 2: Predicted likelihood of hysteranthy across the ﬂowering period of 13 American plum species and the temporal predictors that drive these patterns. Panel a) depicts the predicted likelihood that each species would express hysteranthy on each day of their ﬂowering season based on 1000 draws from the posterior distribution of Bayesian hierarchical models. The colored shapes represent how the likelihood changes over time and the boxed numerical values represent the average likelihood a species would express hysteranthy, summed across the full ﬂowering period. Panel b) depicts the inﬂuence of among season (year of sample) and within season (day of year of sample) trends on the likelihood species would express hysteranthy. Points are the mean eﬀect size estimates, while thick and thin bars represent the 50% and 89% uncertainty intervals respectively.

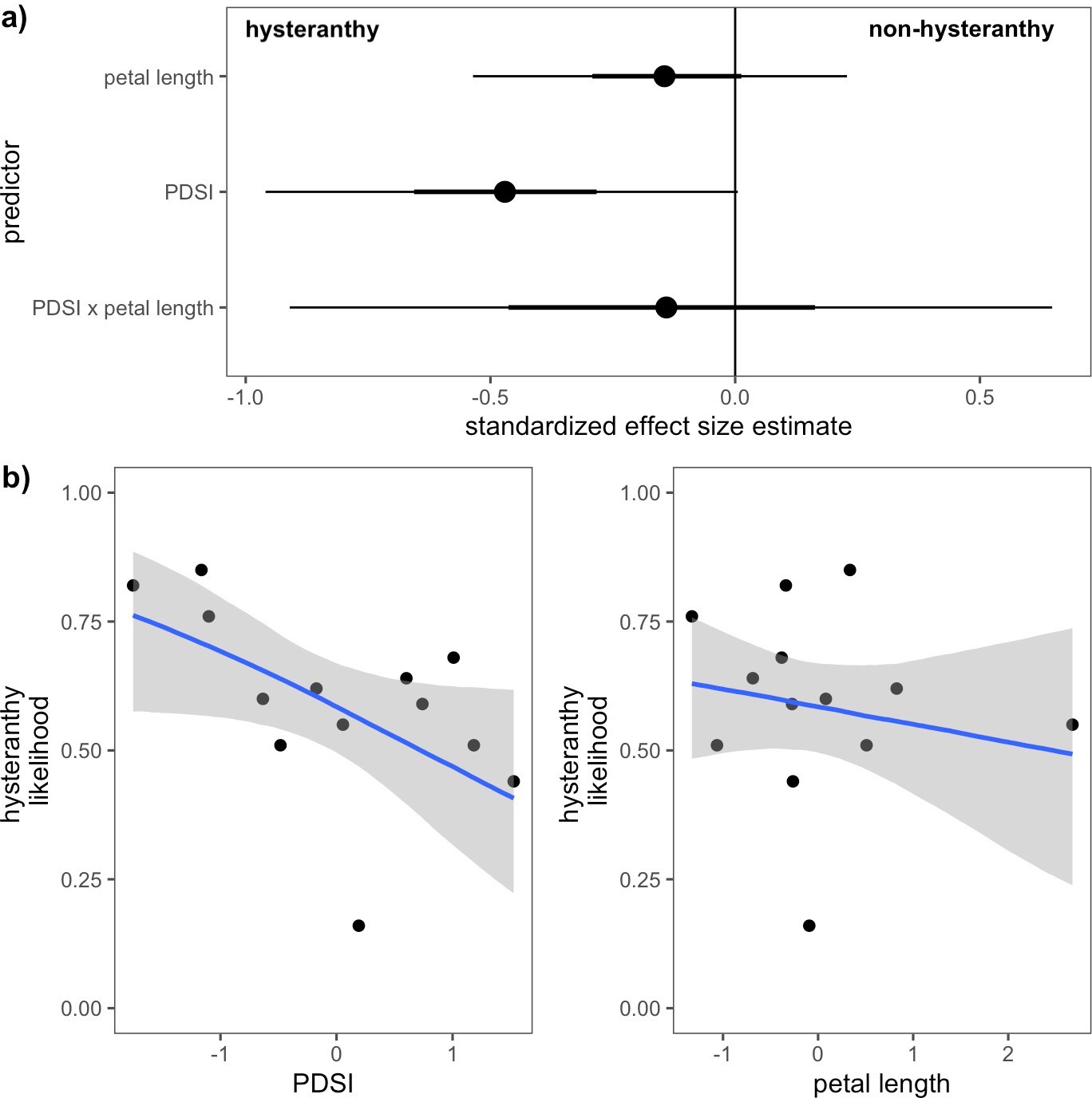


Figure 3: Relationships between hysteranthy and environmental and biological traits for the 13 species of the American Plums. Panel a) shows the estimated eﬀects of each predictor and their interaction on the likelihood hysteranthy. Points indicate the mean eﬀects and the thick and thin bars represent the 50% and 89% uncertainty intervals, respectively. Panel b) depicts the conditional eﬀects of each predictor on hysteranthy likelihood. Blue lines indicate the mean estimate and grey ﬁll the 89% uncertainty intervals. Predictor values (*x*-axis) have been *z*-scored to allow direct comparisons between predictors.

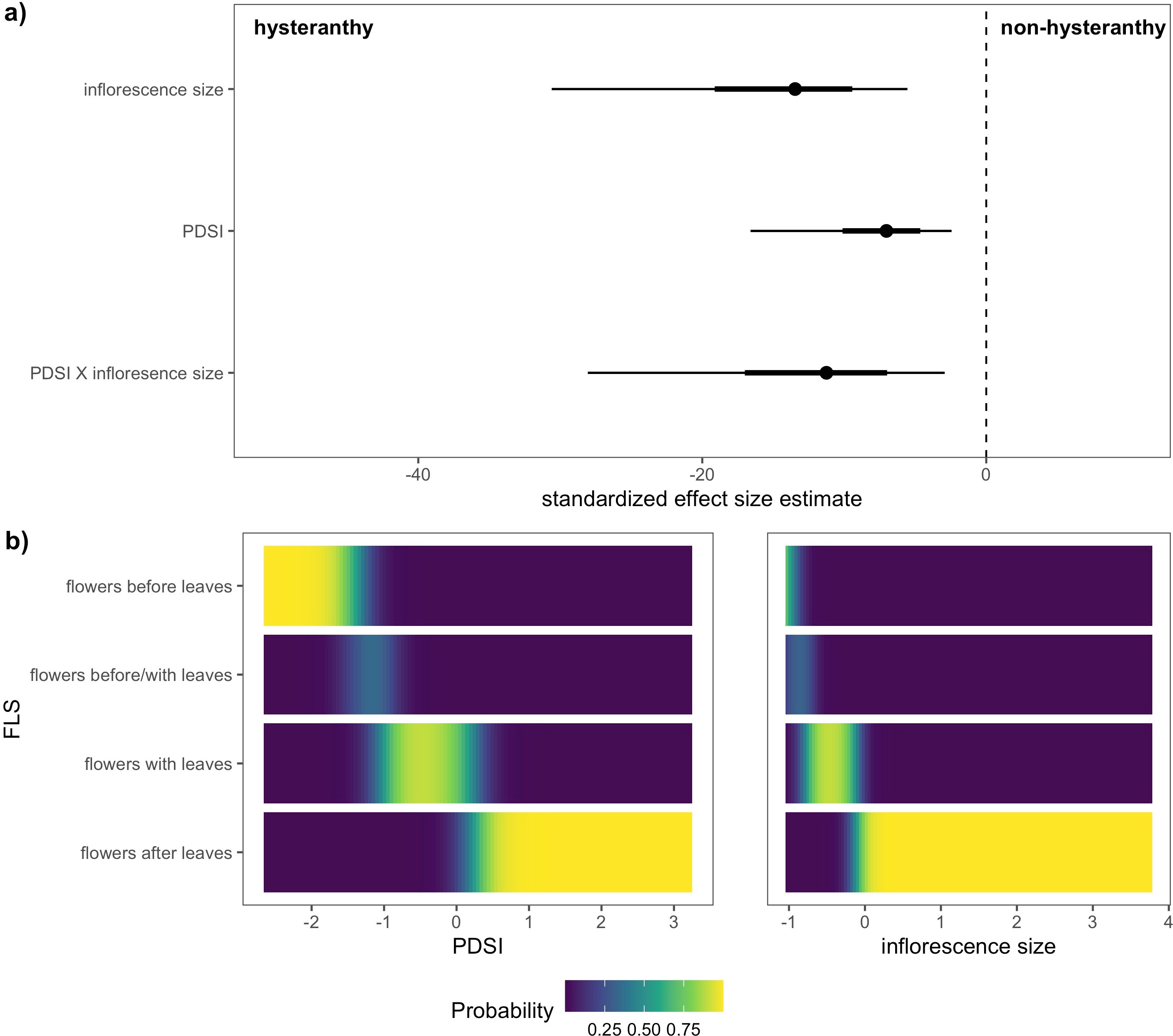


Figure 4: Relationships between the likelihood of hysteranthy and environmental and biological traits for 32 species of the genus *Prunus* native to, or established in North America. Panel a) shows the estimated eﬀect size of each predictor. Points indicate the mean estimate for each predictor, and thick and thin bars the 50% and 89% uncertainty intervals, respectively. Panel b) depicts the likelihood for each ﬂower-leaf sequence stage (*y*-axis) at any given values of PDSI or number of ﬂowers/inﬂorescence (inﬂorescence size). Predictor values (*x*-axis) have been *z*-scored to allow direct comparisons between predictors.