

1           **Environmental influences on the mating system of the common morning glory**

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5

6   **ABSTRACT**

7           The balance between selfing and outcrossing is a life history trait of major concern with  
8 deep evolutionary consequences in mixed mating species. Yet, our current understanding of the  
9 proximate and ultimate determinants of species' mating system is still unsatisfactory and largely  
10 theoretical. Indeed, evolutionary biologists are still puzzled by the often dramatic variation of  
11 mating strategies within single species. Of particular concern is the extent to which  
12 environmental conditions shape patterns of variation and covariation of mating system  
13 components within species. Here, we address this concern in the common morning glory  
14 (*Ipomoea purpurea*) by taking advantage of an extensive dataset of floral traits, genetic estimates  
15 of selfing and inbreeding, and relevant environmental factors compiled for 22 populations of this  
16 species distributed along a disparate set of environments along Southeast and Midwest USA.  
17 Combining a powerful array of parametric and model-free statistical approaches, we robustly  
18 identify a set of natural and anthropogenic environmental factors underlying population-level  
19 variation in selfing, inbreeding, and flower morphology. Remarkably, individual mating system  
20 components are found to be associated with different environmental factors and only loosely  
21 associated with each other, and thus potentially under multiple different selective pressures.  
22 These results not only corroborate theoretical expectations of the significant role the environment  
23 plays in the local determination of mating systems, but also provide compelling evidence of  
24 complex underlying interactions between multiple evolutionary processes.

25

26 **KEYWORDS:** inbreeding, *Ipomoea purpurea*, mating system, morning glory, selfing

## 27 INTRODUCTION

28 Mating systems influence the genetic structure and diversity of populations and thus are a  
29 key component of species' evolutionary dynamics (Darwin, 1876; Charlesworth, 2006). Mating  
30 systems directly impact fitness (Agren and Schemske, 1993) and hence the maintenance of  
31 populations (Pujol *et al.*, 2009), as well as the ability of populations to respond to selection (Noël  
32 *et al.*, 2017). Thus, not surprisingly the diverse number of mating system types among flowering  
33 plants have both interested and puzzled evolutionary biologists for centuries. In particular, the  
34 ability of many species to produce progeny through both selfing and outcrossing (i.e., mixed  
35 mating systems) and the often dramatic variation of selfing and outcrossing rates within single  
36 species have challenged simple evolutionary explanations (Goodwillie *et al.*, 2005; Karron *et al.*,  
37 2012). Several theoretical explanations have been put forward to reconcile these patterns with the  
38 strong fitness consequences that alternative reproductive modes carry for their bearers (Kalisz,  
39 1989; Goodwillie *et al.*, 2005; Glémin *et al.*, 2006). Yet, the question of how mixed mating  
40 systems are maintained and what explains its variability across populations remains open. What  
41 is clear, however, is that underlying these patterns are environmentally driven processes.

42 Multiple environmentally driven processes are undoubtedly acting simultaneously. Their  
43 combined effect on the evolution and maintenance of mixed mating systems depends on their  
44 influence on the trade-off between reproductive assurance and inbreeding depression avoidance  
45 (Lande and Schemske, 1985; Goodwillie *et al.*, 2005; Barrett, 2014). On one hand, local  
46 environmental conditions may limit opportunities for outcrossing (e.g., by reducing the  
47 abundance of pollinators; Scheper *et al.*, 2013; Cusser *et al.*, 2016, or conspecifics; Sagarin *et al.*,  
48 2006). Under these circumstances plants should benefit from being able to produce offspring

49 through selfing (Goodwillie *et al.*, 2005)—a capability that, if not counterbalanced by other  
50 evolutionary forces (Fisher, 1941; Stone *et al.*, 2014), should be favored given the reproductive  
51 assurance it confers. Under these conditions alleles that allow selfing should rapidly increase in  
52 frequency given the automatic transmission advantage of self-fertilization (i.e., the  
53 proportionately higher representation of selfed genes among offspring). On the other hand, if  
54 local environmental conditions do not limit outcrossing opportunities, selfing could be  
55 detrimental as it increases the chances of inbreeding depression and pollen discounting (i.e.,  
56 reduction in the opportunities for pollen to contribute to the outcrossing pollen pool; Chang and  
57 Rausher, 1998; Harder and Wilson, 1998; Fishman, 2000). Together these environmentally  
58 dependent interactions should ultimately determine the specifics of populations' mating system  
59 (Barret and Eckert, 1990).

60         Disentangling environmental drivers of mating systems is, however, remarkably  
61 complicated because environmental factors influence multiple processes. First, local  
62 environmental variation conditions the relative fitness consequences of inbreeding and hence, the  
63 relative cost of selfing (Armbruster and Reed, 2005; Cheptou and Donohue, 2011). Second,  
64 environmental variation determines the opportunities for outcrossing via its influence on both the  
65 extent and effectiveness of pollen movement between and within populations (Friedman and  
66 Barrett, 2009; Cranmer *et al.*, 2012) and by shaping traits that are involved in pollinator  
67 attraction (Totland, 2001; Nicolson and Nepi, 2005). Furthermore, local population sizes and  
68 extirpation probability, and hence the effectiveness of selection, are also dependent on local  
69 environmental variation (Byers and Waller, 1999). In turn, these environmentally driven  
70 interactions are expected to affect a set of diverse evolutionary processes, including i) the sorting  
71 of genetic diversity (Glémin *et al.*, 2006), ii) the interaction between multiple disparate selective

72 pressures (Barrett, 2003; Sargent *et al.*, 2006), iii) the relative risk of maladaptive gene flow  
73 (Peterson and Kay, 2015), and iv) the relative complexity of underlying genetic architecture  
74 (Holtsford and Ellstrand, 1992; Fishman and Stratton, 2004). Further complicating matters, all  
75 these multiple interactions should result in conflicting evolutionary pressures acting on different  
76 mating components (Ivey and Carr, 2005; Dudley *et al.*, 2007). Thus, under potentially weak  
77 underlying genetic correlations between traits (Lankinen *et al.*, 2007; Dudley *et al.*, 2007),  
78 congruent responses among mating system components are expected only under specific  
79 evolutionary scenarios—such as strong correlated selection (Endler, 1995; Armbruster and  
80 Schwaegerle, 1996). Yet, there is currently limited empirical evidence that investigates this  
81 expectation or examines the mechanisms that underlie mating system variation in natural  
82 populations (Lankinen *et al.*, 2016).

83         To improve our understanding of mixed mating systems it is vital to first investigate how  
84 selfing and inbreeding are impacted by environmental factors across geography. Also needed are  
85 studies that investigate how correlations between mating system components may be affected by  
86 environmental variation. Investigating these patterns would lead to a better understanding of the  
87 mechanistic processes involved in the evolution of mating systems and ultimately answer the  
88 question of how similarly do mating system components—such as the selfing rate and inbreeding  
89 coefficient—respond to environmental variation. Here we address this research gap in *Ipomoea*  
90 *purpurea*, a major agricultural weed, using a comprehensive sample of populations distributed  
91 across a significant portion of the species range. We explore spatial patterns of mating system  
92 variation and uncover the environmental factors that may influence mating in this species.  
93 Specifically, we investigate i) whether floral traits are strongly correlated with mating system  
94 parameters, ii) whether population variation of mating system parameters and their correlation

95 with floral traits is geographically structured, iii) which environmental factors best predict  
96 individual variation of mating system parameters, and iv) whether strength of the floral-mating  
97 system correlation is influenced by the environment. By addressing these questions, we offer  
98 valuable insights into the proximate determinants of mating system variation and their complex  
99 interaction, and provide compelling evidence of the complex nature of the selective pressures  
100 acting on mating systems.

101

## 102 **METHODS**

### 103 **Study system**

104 Our study focuses on *I. purpurea*, a climbing annual vine with a wide distribution across  
105 both Central and North America (Ennos, 1981; Defelice, 2001). Appreciated in horticulture for  
106 its colorful flowers, this species has become a weed of major agricultural concern worldwide  
107 (Baucom and Mauricio, 2004; Fang *et al.*, 2013). *I. purpurea* shows geographic variability in  
108 both selfing rates (Kuester *et al.*, 2017) and resistance levels to glyphosate—the most commonly  
109 used herbicide in the US (Benbrook, 2016)—with some populations exhibiting 100% survival  
110 after application of standard recommended doses of glyphosate and other populations exhibiting  
111 high susceptibility (Kuester *et al.*, 2015). Previous research on this hermaphroditic species has  
112 identified heritable variation in anther-stigma distance (Chang and Rausher, 1998), a trait that  
113 has consistently been found to be linked to selfing rate (Holtsford and Ellstrand, 1992; Duncan  
114 and Rausher, 2013), as well as a significant association between resistance to glyphosate and  
115 outcrossing rates (Kuester *et al.*, 2017). This species is thus particularly suitable for investigating  
116 the role of both natural and anthropogenic environmental variation in the maintenance of mixed  
117 mating systems. Here, we focused on 22 populations of *I. purpurea* sampled along a disparate set

118 of environments along Southeast and Midwest USA in 2012 (Fig. 1), and addressed the extent to  
119 which mating system trait variation and covariation are impacted by natural and anthropogenic  
120 environmental factors.

121

## 122 **Data compilation**

123 To characterize the environments of the 22 sampled populations of *I. purpurea*, we  
124 compiled data on a wide range of abiotic factors that could (directly or indirectly) influence  
125 mating system variation in this species (Table S1). Given the difficulty of measuring all these  
126 environmental factors *in situ*, we chose to use remote sensing and census data. While this  
127 decision carries an intrinsic spatial resolution limitation (Holtsford and Ellstrand, 1992), GIS  
128 data at moderate to coarse resolutions have been shown to reasonably capture biologically  
129 relevant population-level processes (Kerr and Ostrovsky, 2003; Kozak *et al.*, 2008). In addition,  
130 because of the previously identified association between resistance to glyphosate and outcrossing  
131 rates (Kuester *et al.*, 2017), we included population-level glyphosate resistance estimates—  
132 measured as the proportion of surviving individuals after the application of manufacturer’s  
133 recommended doses of glyphosate (Kuester *et al.*, 2015), along with county-level estimates of  
134 the cumulative amount of glyphosate applied to these populations over the last two decades  
135 (years 1992–2012). Unfortunately, no reliable data on bumblebees’ abundance—*I. purpurea*’s  
136 primary pollinators (Defelice, 2001)—could be found to be included in our analyses. Our  
137 complete environmental dataset included a total of 31 predictor variables (Table S1) with several  
138 that were highly correlated with each other. Therefore, we performed a hierarchical  
139 agglomerative clustering in R3.3.3 (R Core Team, 2017), using package ClustOfVar (Chavent *et*  
140 *al.*, 2013) to select a non-redundant set of environmental predictors. This analysis clusters

141 variables into statistically homogeneous sets and hence identifies groups of variables that  
142 basically bring the same information (Chavent *et al.*, 2012). We chose this analysis because it  
143 has the advantage of interpretability over alternative approaches such as principal component  
144 analysis (Dormann *et al.*, 2013). By selecting from each resulting cluster the variable less  
145 correlated with the other clusters, we retained a set of 8 non-highly-correlated environmental  
146 variables (average absolute Pearson's coefficient = 0.36 [0.01–0.74]).

147 In addition to the environmental data, a set of four floral measurements were taken over  
148 multiple dates in the fall of 2014 from a total of 445 individuals from all 22 populations grown at  
149 the Matthaei Botanical Gardens at the University of Michigan (Ann Arbor, MI, USA) (Kuester *et*  
150 *al.*, 2017). Specifically we measured the length of the tallest stamen to the top of the anther  
151 (TAL), height of the pistil to the top of the stigma (SL) and the length (CL) as well as width  
152 (CW) of the corolla (Fig. 1) on multiple flowers per individual (median number of flowers per  
153 individual: 4 [1–25]; median number of individuals per population: 15 [3–23]). In addition, we  
154 calculated the difference between the length of the tallest stamen and the height of the pistil, or  
155 the anther-stigma distance (ASD). All five floral traits were averaged for each population across  
156 flowers, dates, and individuals. Finally, because all four averaged floral measurements (i.e.,  
157 TAL, SL, CL, CW) were highly correlated with each other (Fig. S1a), we condensed them into a  
158 single variable by running a principal component analyses on their covariance matrix after  
159 scaling all of them. The retained first principal component, which accounted for 73.77% of the  
160 total variance was equitably negatively associated with all four floral measurements included  
161 (Fig. S1b), and primarily summarized overall flower size (with lower scores corresponding to  
162 bigger flowers). For ease of interpretation, however, populations' scores on this axis were

163 multiplied by -1 so that flower size increased as PC scores increased. The resulting inverted axis  
164 was used as an additional covariate of mating system traits in subsequent analyses.

165 We quantified mating system estimates for the 22 populations with floral data using  
166 individuals genotyped at 15 microsatellite loci previously developed for *I. purpurea* (Aksoy *et al.*  
167 *al.*, 2013). Specifically, we analyzed this dataset, which included 4584 genotyped individuals  
168 (median number of individuals per population: 207 [29–417]), in both BORICE (Koelling *et al.*,  
169 2012) and MLTR (Ritland and Jain, 1981; Ritland, 2002) with default parameters. Because  
170 BORICE and MLTR estimates were correlated with each other and BORICE is known to  
171 outperform MLTR when maternal genotypes are unavailable (Koelling *et al.*, 2012), we kept  
172 only BORICE's estimates of the family-level outcrossing rate ( $t$ ) and maternal-line inbreeding  
173 coefficients ( $F$ ) for all subsequent analyses. It is important to note that because removing loci  
174 identified as having over 25% null alleles using Micro-Checker (Van Oosterhout *et al.*, 2004) did  
175 not significantly impact BORICE's estimates, we opted for  $F$  and  $t$  estimates based on all 15 loci  
176 (Kuester *et al.*, 2017). We also calculated inbreeding depression ( $\delta$ ) using Ritland's (Ritland,  
177 1990) formula under the assumptions that populations are at inbreeding equilibrium and that the  
178 genetic markers used are effectively neutral (Ritland, 1990; Goodwillie *et al.*, 2005).

179

## 180 **Trait correlation**

181 To investigate the degree of correlation between our floral traits and mating system  
182 parameters we chose to run separate analyses for our composite floral trait (i.e., ASD) and for  
183 our direct floral measurements (TAL, SL, CL, and CW). We made this decision because of the  
184 likely cause-effect relationship of ASD with selfing rates (Chang and Rausher, 1998) and the  
185 lack of significant correlations between ASD and the other floral traits, albeit the remarkably



186 high correlations among all four direct measurements (Fig. S1). First, we calculated pairwise  
187 Pearson's simple correlation coefficients between ASD and  $t$ ,  $F$  and  $\delta$ . In addition, we explored  
188 the degree of multivariate correlation between our four direct floral measurements (TAL, SL,  
189 CL, and CW) and all three mating system parameters ( $t$ ,  $F$ ,  $\delta$ ) by running a canonical correlation  
190 analysis (CCA) in R3.3.3 (R Core Team, 2017) using package CCA (González and Déjean,  
191 2012). This latter analysis identifies a set of axes that maximize the correlation between two sets  
192 of variables (floral and mating system variables in our case) and hence quantifies the extent and  
193 significance of their multivariate relationship (Hotelling, 1936). For subsequent analyses we kept  
194 the first pair of CCA axes, which as expected shows the strongest correlation.

195

## 196 **Geographic structure**

197 To assess how mating strategies are structured in *I. purpurea* populations over our study  
198 area, we investigated how mating system parameters as well as their correlation with floral traits  
199 vary based on geographic location. First, we obtained proxies for floral-mating system  
200 correlation strength for each population by rerunning the correlation analyses described in the  
201 previous section in a leave-one-out manner. That is, we recalculated the Pearson's and CCA  
202 correlation coefficients after removing each population in turn. We then used the difference  
203 between the absolute values of the leave-one-out correlation estimate and the all-data correlation  
204 estimate as our index of local population correlation strength ( $\lambda$ , hereafter). Positive values of  
205 this index (leave-one-out estimate > all-data estimate) indicate a stronger local association than  
206 the global association, whereas negative values (leave-one-out estimate < all-data estimate)  
207 indicate a weaker local association. Next, we assessed the association of both, individual  
208 parameters and  $\lambda$ s, with geographic location by running independent multivariate linear

209 regressions for each element or  $\lambda$  against longitude and latitude. In addition, we assessed the  
210 degree of global and local spatial autocorrelation on these data by calculating global and local  
211 Moran's I (Moran, 1950; Anselin, 1995) in R3.3.3 (R Core Team, 2017) using packages ape  
212 (Paradis, 2011) and spdep (Bivand and Piras, 2015), respectively. For the local analysis, we  
213 adjusted p-values for multiple comparisons using a (Benjamini and Hochberg, 1995) false  
214 discovery rate method.

215

### 216 **Environmental influence on individual trait variation**

217 To examine the relationship between the 8 selected environmental factors and individual  
218 mating system traits, we ran Ordinary Least Squares (OLS) multivariate linear regressions for  
219 each mating system trait and performed backward stepwise variable selection using a resampling  
220 model calibration strategy with 500 bootstrap replicates. This strategy allows for bias-correction  
221 of error estimates based on nonparametric smoothers and hence avoids possible overfitting given  
222 our sample size (Harrell, 2015). All these analyses were run on standardized environmental  
223 variables using the package rms (Harrell, 2017) in R3.3.3 (R Core Team, 2017). In addition, we  
224 estimated the relative contribution of each predictor retained by comparing their associated  
225 regression coefficients (i.e., the expected change in the independent variable per unit change of a  
226 predictor when all other predictors in the model remain constant). Given our sample size,  
227 however, no interactions were included in any model.

228 Because several OLS assumptions might be violated by our dataset we additionally  
229 chose to run homologous model-free regressions using machine-learning tools. Specifically, we  
230 opted to run Random Forest (RF) regressions because they deal efficiently with i) the large p-  
231 small n problem (large number of predictors relative to observations), ii) non-linear relationships

232 between independent and predictor variables, and iii) predictors multicollinearity (Breiman,  
233 2001; Strobl *et al.*, 2008). RF regressions use an ensemble of multiple-regression trees to fit  
234 subsets of the data onto the different predictors by minimizing the sum of square errors and  
235 summarize this ensemble of trees by bootstrapped aggregation (a.k.a. bagging; Breiman, 1999).  
236 To select the best set of predictors in these regressions, we iteratively fitted the RF algorithm by  
237 removing in each iteration the environmental predictor variable with the unscaled smallest  
238 variable importance (i.e., backwards variable selection) until the out-of-bag error (i.e., error rate  
239 from samples not used in the construction of a given tree) did not decrease any further (Díaz-  
240 Uriarte and Alvarez de Andrés, 2006; Strobl *et al.*, 2008). We chose this greedy method of  
241 variable selection because an exhaustive search over all possible subsets of predictors is  
242 computationally prohibitive. The relative contribution of predictors in the final model was then  
243 assessed by the unscaled increase in the mean square error (MSE) of the prediction after removal  
244 of each variable (Liaw and Wiener, 2002; Strobl *et al.*, 2008). Finally, the respective functional  
245 relationship of each predictor with the independent variable was recovered using partial  
246 dependency plots [Citation error], which are graphical visualizations of the marginal effect of a  
247 given variable when the other variables are kept constant. All these analyses were run in R3.3.3  
248 (R Core Team, 2017) using package randomForest (Liaw and Wiener, 2002).

249

## 250 **Environmental influence on traits correlation**

251 Finally, we evaluated how the univariate trait correlation was impacted by environmental  
252 variation by re-calculating Pearson's correlation coefficients on subsamples of populations  
253 grouped according to their environmental variable values. We followed a similar procedure re-  
254 estimating the multivariate trait correlation by re-running the CCA analysis on environmentally

255 grouped sets of populations. Specifically, we independently grouped each environmental variable  
256 into quantiles and used these groups to split our set of populations based on similarity on each  
257 environmental variable. We then separately calculated the simple and CCA correlation  
258 coefficients between floral traits and mating system parameters (as done above) for each  
259 subsample. We assessed the significance of the effect of this environmental grouping by  
260 comparing the correlation coefficient obtained against similarly obtained coefficients from a set  
261 of 100 randomly split datasets that share the same number of observations for each split as the  
262 environmentally grouped data.

263

## 264 **RESULTS**

265 **Trait correlation**--As previously found (Chang and Rausher, 1998), ASD was  
266 significantly correlated with outcrossing rate ( $t$ ) (Fig. 2). Yet, ASD was not significantly  
267 correlated with either inbreeding coefficient of maternal individuals ( $F$ ) or inbreeding depression  
268 ( $\delta$ ) in our dataset. All other floral traits were correlated in a multivariate manner with these three  
269 mating system parameters, although the canonical axes themselves were not significant (Table  
270 S2). In this latter analysis, corolla (CL) and tallest stamen (TAL) lengths, which are  
271 themselves significantly correlated with each other (Fig. S1), showed the strongest effect on  
272 mating system parameters. With other floral variables held constant, increments in CL were  
273 associated primarily with an increase in  $t$  and a decrease in  $\delta$ , whereas increments in TAL had a  
274 slightly weaker but opposite effect.

275

276 **Geographic structure**--We did not uncover significant geographic structure across  
277 populations for ASD, for the three examined mating system parameters, or for their correlation

278 with floral traits in *I. purpurea*. No mating system component or floral-mating system correlation  
279 index ( $\lambda$ ) was significantly associated with either latitude or longitude (Fig. S2), and we did we  
280 not find evidence of spatial autocorrelation, as measured by global Moran's I, in any of our  
281 individual variables or  $\lambda$ s (Fig. 3). In addition, no population exhibited local spatial  
282 autocorrelation in any of our traits (Table 1), meaning there was no evidence for geographically  
283 proximate populations showing similar trait values in the study range examined. Further, there  
284 was no evidence of local spatial autocorrelation in the correlation indexes (Table 1).

285

286 **Environmental influence on individual trait variation--**Both OLS and RF regressions  
287 identified unique sets of environmental predictors for the different components of *I. purpurea*'s  
288 mating system (Tables 2 and 3). Despite the different statistical approaches (Breiman, 2001),  
289 resulting in different total number of predictors retained (being in most cases greater in the RF  
290 regressions; Tables 2 and 3), both methods robustly identified the same top predictors for all four  
291 mating system parameters as well as a relatively congruent relationship between predictors and  
292 parameters (Figs. S3 and S4). For example, relative humidity, which was identified as the  
293 stronger predictor of ASD under both methods, showed an inversely proportional relationship  
294 with ASD in the OLS regression (Fig. S3a) and a thresholded negative association with ASD in  
295 the RF regression (Fig. S4a). Across both sets of regressions, we found that herbicide resistance  
296 (survival rate after glyphosate application) was the most common predictor (retained in 5 out of  
297 8 regressions), explaining in some cases up to 34% of the variance in mating system traits  
298 (Tables 2 and 3). The second most common predictor of mating system traits in our analyses was  
299 temperature range (retained in 4 out of 8 regressions), which explained up to 21% of the  
300 variance. Nonetheless, the relative importance of these factors—measured as the net effect that

301 change in the predictor causes in the response trait (OLS) or as the increase in the prediction  
302 error the predictor removal causes (RF)—vary significantly across mating system traits (Tables 2  
303 and 3). For instance, in both OLS and RF regressions annual temperature range was identified as  
304 the most important predictor of inbreeding depression ( $\delta$ ), but only as the third most important  
305 predictor of inbreeding coefficient (F). Likewise, the shape of their relationship varies across  
306 mating system parameters in an independent manner. For example, whereas the inbreeding  
307 coefficient (F) showed a negative relationship with annual temperature range (Figs. S3c and  
308 S4c), inbreeding depression ( $\delta$ ) showed a positive association with this environmental factor  
309 (Figs. S3d and S4d).

310 In summary, the regressions identified idiosyncratic environmental associations for the  
311 four mating system parameters analyzed. ASD decreased as relative humidity increased,  
312 elevation and mean temperature increased (OLS only), and that it peaks at intermediate values of  
313 annual precipitation and herbicide resistance (RF only) (Figs. S3 and S4). In contrast, as  
314 previously observed (Kuester *et al.*, 2017), outcrossing rate is primarily affected by herbicide  
315 resistance, being the lowest at higher resistance values (soil coarseness and herbicide use are  
316 comparatively minor predictors in RF; Tables 2 and 3). Similarly, the inbreeding coefficient was  
317 strongly associated with herbicide resistance and proportionally increased as resistance  
318 increased, as previously described (Kuester *et al.*, 2017). The inbreeding coefficient (F) was also  
319 positively associated to mean temperature (OLS only) and relative humidity (RF only), and  
320 inversely associated to annual precipitation (OLS only) and annual temperature range (OLS and  
321 RF) (Tables 2 and 3). On the other hand, inbreeding depression was primarily explained by  
322 annual temperature range (Tables 2 and 3) and showed a positive relationship with this  
323 environmental factor. It is important to note however, that most other factors analyzed were

324 retained in the RF regression for this latter trait; yet, the overall predictive power of this RF  
325 regression was relatively low. Thus, while we found significant associations between  
326 components of the mating system and environmental factors, the relationships between these  
327 components and environment factors was not congruent.

328

329 **Environmental influence on trait correlation**--Analyses on how the floral-mating  
330 system correlations varied across environmental gradients indicated that no natural  
331 environmental factor significantly influenced the univariate correlation between ASD and t, F or  
332  $\delta$  (results not shown). Nor was there any significant association between natural environmental  
333 factors and the multivariate correlation between our floral morphological measurements (TL, SL,  
334 CL, and CW) and mating system parameters (results not shown). In contrast, herbicide resistance  
335 and herbicide use significantly impacted the correlation between ASD and outcrossing rate and  
336 ASD and inbreeding coefficient, respectively. Specifically, the ASD-t correlation was stronger at  
337 the greatest herbicide resistance values (Fig. 4a), whereas the ASD-F correlation was weaker at  
338 moderately high herbicide use (Fig. 4b). No significant effect of either herbicide use or herbicide  
339 resistance on the univariate correlation between ASD and inbreeding coefficient (Fig. 4c) or the  
340 multivariate correlation between floral and mating system parameters was recovered (Fig. 4d).

341

## 342 **DISCUSSION**

343 Our study identifies disparate environmental factors that influence variation in the mating  
344 system of *I. purpurea* across a significant portion of its range. While ASD and the level of  
345 inbreeding depression were primarily associated with natural environmental variation, the  
346 outcrossing rate and inbreeding coefficient were most strongly associated with the level of

347 herbicide resistance (Kuester *et al.*, 2017). The selection pressure imposed by herbicide use also  
348 seems to influence the strength of the association between ASD and selfing and inbreeding.  
349 Particularly noteworthy, we did not recover any other environmental influence on the overall  
350 weak association between outcrossing rate, inbreeding coefficient, and inbreeding depression  
351 with floral traits (TAL, SL, CL, CW, and ASD). Further, we did not find significant geographic  
352 structure in any of the mating system parameters explored or their inter-correlation. Taken  
353 together, these results suggest that different components of *I. purpurea*'s mating system are  
354 presumably under multiple different selective pressures, and that parameters of the mating  
355 system and floral traits in this species are not tightly linked. Importantly, these results highlight  
356 the complex influence of environmental factors on the mating system of this agricultural weed,  
357 and show that human influence is currently a major component of the selfing/outcrossing balance  
358 across its populations.

359

### 360 **The complexity of mating systems**

361 Compelling empirical evidence supports an association between individual mating  
362 system components and environmental conditions, such as the one found here. For example,  
363 outcrossing rate has been found to covary in a variety of plant systems with elevation (Neale and  
364 Adams, 1985), humidity (Brown *et al.*, 1978; Shea, 1987), and temperature (Holtsford and  
365 Ellstrand, 1992). Similarly, ASD has been found to strongly respond to environmental factors,  
366 including humidity (Elle and Hare, 2002; Van Etten and Brunet, 2013), water and nutrient  
367 availability (Vallejo-Marín and Barrett, 2009), light regime (Brock and Weinig, 2007), and  
368 temperature (Lankinen *et al.*, 2016). Also in agreement with our findings, plenty of studies have  
369 identified an association between ASD (herkogamy) and outcrossing rates (Chang and Rausher,



1998; Motten and Stone, 2000; Takebayashi *et al.*, 2006; but see Medrano *et al.*, 2005), and some have identified associations between ASD and inbreeding depression within individual populations (Takebayashi and Delph, 2000; Stone and Motten, 2002; but see Carr *et al.*, 1997). Yet, a relatively small number of studies have simultaneously explored variation patterns of multiple mating system parameters in natural populations across environmental gradients. Among those that have, a variable strength of association is often identified (e.g., Lankinen *et al.*, 2016), which has prompted the hypothesis that variation in the different mating system parameters, such as selfing and inbreeding depression, is more strongly conditioned by other factors (e.g., population size and intraspecific competition for pollinators) than by each other (Johnston and Schoen, 1996; Spigler *et al.*, 2010). Our findings support this hypothesis of relative independence of mating system components (Johnston and Schoen, 1996; Dudley *et al.*, 2007) as well as its expectation of limited cohesive responses among mating system components to environmental variation. The idiosyncratic responses recovered also suggest that mating system determination is very complex and driven by multiple interacting processes. Yet, it remains to be investigated what those interacting processes are and how they are (directly or indirectly) shaped by environmental factors.

Further attesting the complexity of mating system variation is the lack of geographic structure recovered across all mating system parameters. This highlights the importance of fine-tuning mating strategies (through plasticity and/or adaptation) to local environmental conditions. Considering the dramatic evolutionary consequences that reproductive strategies may carry (Kalisz, 1989; Glémin *et al.*, 2006), individuals with reproductive strategies ill-matched to their environmental reality are expected to experience strong detrimental fitness consequences. In line with this expectation, population-level differences in selfing rates are usually associated with

393 habitat quality, as pollen flow is more limited in harsher habitats (Griffin and Willi, 2014; Matos  
394 Paggi *et al.*, 2015). More generally, the lack of geographic structure also highlights the  
395 importance of local interactions between multiple environmental factors and hence, the complex  
396 nature of environments plants have to interact with (Holtsford and Ellstrand, 1992; Sagarin *et al.*,  
397 2006).

398         At least in *I. purpurea*, there is a combination of multiple different environmental factors  
399 acting on different components of its mating system. Inbreeding coefficient, for example, is  
400 associated with both climatic variation and herbicide selective pressure (see below). Inbreeding  
401 depression is instead mostly explained by annual temperature range, with stronger depression in  
402 more temperature-seasonal environments, raising the possibility of additional environmentally  
403 driven processes contributing to determine the fitness consequences of inbreeding. For instance,  
404 it is possible that the strong association with temperature seasonality, which is a proxy for  
405 environmental stability, reflects the more detrimental effect of inbreeding in more stressful  
406 environments (Armbruster and Reed, 2005; Cheptou and Donohue, 2011). In contrast, the  
407 negative association between ASD and relative humidity might be best explained by selection for  
408 reproductive assurance as pollinators' visitation rates have been found to decrease with relative  
409 humidity at least in some plant species (Wang *et al.*, 2009). While these hypotheses definitively  
410 require further testing, especially considering the impossibility of assessing direct causality from  
411 statistical associations (Mac Nally, 2000), the pattern of differential responses to environmental  
412 factors that emerges unequivocally demonstrates the complex integration of mating system  
413 strategies and calls for the inclusion of biologically realistic complexity in mixed mating system  
414 models.

415

## 416 **The pervasive role of humans**

417           While a significant association between herbicide resistance and selfing rate has been  
418 previously uncovered in *I. purpurea* (Kuester et al., 2017), it remained unclear the relative  
419 importance of this association in relation to other environmental factors or whether this was a  
420 spurious association mediated by other environmental factors (herbicide resistance is itself  
421 correlated with precipitation and soil variables; Alvarado-Serrano and Baucom unpublished  
422 data). It was also unknown if herbicide resistance similarly impacted other mating system  
423 components in this species and hence, what is the overall impact of herbicide usage on its  
424 reproductive strategies. Here, by simultaneously exploring the association of mating system  
425 components with multiple environmental variables, we provide further support to the hypothesis  
426 that anthropogenic-driven selection indeed plays a major role in *I. purpurea*'s mating system  
427 dynamics. *Ipomoea purpurea*'s response to the continuous application of glyphosate is by far the  
428 strongest predictor of outcrossing and inbreeding rates, and it has a significant impact on the  
429 association of these traits and ASD. Specifically, our results suggest that ongoing selection for  
430 herbicide resistance in this species may simultaneously favor increased selfing rate and the  
431 inbreeding coefficient, promoting a stronger link between them (Fig. 4). This is because the  
432 strong selective pressure imposed by herbicide application presumably reduces the number of  
433 conspecifics available to mate with (favoring shorter ASD and increased selfing) and also  
434 increases the fitness costs of mating with non- or less-resistant plants (favoring inbreeding).  
435 Under these circumstances, other (arguably weaker) selective forces normally acting rather  
436 independently on different mating system components might be superseded by the remarkably  
437 strong selection imposed by herbicide use (Culpepper *et al.*, 2001). In this way, herbicide  
438 application is expected to also impact the evolutionary trajectories of weeds by favoring stronger

439 floral integration for increased selfing (Rosas-Guerrero *et al.*, 2011; Fornoni *et al.*, 2016), and by  
440 altering the overall genetic constitution of populations (through inbreeding).

441         Although the impact of human activities on world's ecosystems is undeniable (Vitousek  
442 *et al.*, 1997; Haberl *et al.*, 2007), limited evidence exists of their influence on mixed mating  
443 systems in natural populations. The available evidence mostly supports an indirect impact of  
444 human activities on mating systems, mediated by habitat modifications (Eckert *et al.*, 2010). Yet,  
445 examples of human activities directly conditioning selfing and inbreeding rates and related  
446 phenotypic traits remain less common. Our findings not only highlight the extensive cascade of  
447 consequences of human activities on species, but also provide further support for humans as  
448 direct selective agents of mating strategies (Kuester *et al.*, 2017). While it remains to be seen  
449 how prevalent these effects are in less extreme selective regimes, our findings reveal the high  
450 evolutionary lability of mating systems and hence its potential sensibility to anthropogenic  
451 impacts. Specially considering the relatively short time scale over which it has happened in *I.*  
452 *purpurea* (Duke and Powles, 2008), our results call attention to the need of considering the  
453 potential major impact of human-driven selection on such a fundamental life history trait in  
454 management and conservation efforts.

455

#### 456 **Future directions and conclusions**

457         Although years of research on mixed mating systems have identified a plethora of  
458 plausible mechanisms underlying its maintenance (Barrett, 2014), there has been seldom  
459 empirical evidence to assess their relative contribution in natural populations. Our unique  
460 dataset, which comprises a rare combination of floral measurements and mating system estimates  
461 over a significant portion of a species' range, allows exploring the potential of some of these

462 proposed mechanisms to explain mating strategies. In particular, this unique dataset in  
463 combination with a set of robust statistical analyses unequivocally shows that floral traits and  
464 mating system estimates do not tightly covary, and seem to respond to different environmental  
465 predictors. This finding supports the existence of an intricate network of interactions between  
466 mating system components and environmental variation. The uncovering of such complex  
467 interactions calls for future studies focused on disentangling the specific nature of the recovered  
468 associations to identify direct or indirect causal links between environmental predictors and  
469 species' selfing/outcrossing balance. Yet, even as this work is in progress, the results from the  
470 current study offer practical information to forecast plausible consequential responses of species  
471 to environmental change, a topic of major importance given the significant evolutionary  
472 consequences of variation in mating system patterns.

473

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477

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482

#### 483 **DATA AVAILABILITY**

484 All original data will be made available in Dryad upon acceptance.

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704 **TABLES AND FIGURES**

705 **Table 1.** Summary of Local Moran's I analyses on the extent of local spatial correlation among  
706 mating system traits and floral-mating system covariation ( $\lambda$ ).

707

708 **Table 2.** Summary of Ordinary Least Square linear regressions of mating system parameters on  
709 environmental variables. Results obtained after 1000-bootstrapped backward selection are  
710 presented. Corresponding simple linear regression results are given in Table S3.

711

712 **Table 3.** Summary of Random Forest regressions of mating system parameters on environmental  
713 variables. Only results obtained after recursive backward selection are presented.

714

715 **Figure 1.** Samples distribution of *I. purpurea* populations with floral measurements and mating  
716 system estimates. The four floral measurements taken are shown in upper-right inset: 1) length of  
717 the tallest stamen to the top of the anther, 2) height of the pistil to the top of the stigma, 3)  
718 corolla length, 4) corolla width.

719

720 **Figure 2.** Covariation between floral traits and mating system parameters. a) anther-stigma  
721 distance (ASD) and outcrossing rate ( $t$ ), b) ASD and inbreeding coefficient ( $F$ ), c) ASD and  
722 inbreeding depression ( $\delta$ ), d) first canonical correlation axes of floral and genetic components.

723

724 **Figure 3.** Geographic variability in mating system parameters and their covariation with floral  
725 traits. a) anther-stigma distance (ASD), b) outcrossing rate ( $t$ ), c) inbreeding coefficient ( $F$ ), d)

726 inbreeding depression ( $\delta$ ), e) ASD-t  $\lambda$ , f) ASD-F  $\lambda$ , g) ASD- $\delta$   $\lambda$ , h) CC1  $\lambda$ . Global Moran's I  
727 estimates and associated p-values, after correction for multiple testing, are reported.

728

729 **Figure 4.** Covariation of floral traits and mating system parameters across anthropogenic  
730 selection regimes. Estimates of Pearson's (a-c) and CCA (d) correlation coefficient across  
731 environmentally binned samples are shown by circles, whereas a dashed horizontal line indicates  
732 the global estimate. Grey vertical lines indicate 95% confidence estimates based on random  
733 resampling. Significant estimates are highlighted by brighter symbols.

734 **Table 1.**

<b>Variable</b>	<b>Local Moran's I mean</b>	<b>Standard deviate Local Moran's I</b>	<b>p value range</b>
ASD	-0.132 [-1.160 – 0.52]	-0.250 [-3.598 – 1.278]	0.503 – 1.000
t	-0.197 [-1.078 – 0.813]	-0.397 [-3.139 – 1.917]	0.138 – 1.000
F	-0.048 [-0.572 – 0.921]	-0.022 [-1.534 – 1.848]	0.129 – 1.000
$\delta$	0.113 [-0.512 – 0.888]	0.394 [-1.234 – 2.170]	0.075 – 1.000
ASD-t $\lambda$	0.219 [-1.215 – 0.198]	-0.404 [-2.630 – 0.798]	1.00 – 1.000
ASD-F $\lambda$	-0.069 [-0.880 – 0.860]	-0.116 [-2.848 – 1.881]	0.12 – 1.000
ASD- $\delta$ $\lambda$	0.000 [-0.949 – 0.400]	0.146 [-2.264 – 1.452]	0.50 – 1.000
CC1 $\lambda$	-0.146 [-1.459 – 0.425]	-0.320 [-4.995 – 1.218]	0.586 – 1.000

735

736 **Table 2.**

<b>Dependent</b>	<b>LR<sup>(1)</sup> X<sup>2</sup></b>	<b>p value</b>	<b>R<sup>2</sup></b>	<b>Retained predictors<sup>(2)</sup></b>	<b>Beta coefficients<sup>(3)</sup></b>
ASD	9.11 (3, 18)	0.028	0.34	Elevation	-0.064 (±0.026)
				<b>Relative humidity</b>	-0.052 (±0.018)
				Mean temperature	-0.049 (±0.022)
t	9.05 (1, 20)	0.002	0.34	<b>Herbicide resistance</b>	-0.078 (±0.024)
F	18.44 (4, 17)	0.001	0.57	<b>Herbicide resistance</b>	0.059 (±0.014)
				Annual precipitation	-0.039 (±0.018)
				<b>Temperature range</b>	-0.038 (±0.018)
				Mean Temperature	0.037 (±0.019)
Delta	5.12 (1, 20)	0.024	0.21	<b>Temperature range</b>	0.113 (±0.049)

- 737 1. Model likelihood ratio Chi-square statistic. Degrees of freedom are below in parentheses  
 738 2. Predictors are ordered by absolute beta coefficient magnitude. Predictors also identified  
 739 in Random Forest regressions are in bold.  
 740 3. Standard errors are in parentheses underneath.



741 **Table 3.**

Dependent	MSE <sup>(1)</sup>	% Variance explained	Pseudo-R <sup>2</sup>	Retained predictors <sup>(2)</sup>	Variable importance <sup>(3)</sup>
ASD	3.67e <sup>-3</sup>	24.64	<b>0.50</b> (0.02)	<b>Relative humidity</b> Annual precipitation Herbicide resistance	1.18e <sup>-3</sup> 0.89e <sup>-3</sup> 0.44e <sup>-3</sup>
t	9.26e <sup>-3</sup>	46.62	<b>0.69</b> (<0.01)	<b>Herbicide resistance</b> Soil coarseness Herbicide rate	11.77e <sup>-3</sup> 1.72e <sup>-3</sup> 0.82e <sup>-3</sup>
F	4.76e <sup>-3</sup>	11.55	0.35 (0.11)	<b>Herbicide resistance</b> Relative humidity <b>Temperature range</b>	0.88e <sup>-3</sup> 0.64e <sup>-3</sup> 0.41e <sup>-3</sup>
Delta	53.96e <sup>-3</sup>	7.65	0.28 (0.21)	<b>Temperature range</b> Flower size Herbicide use Soil coarseness Mean temperature Elevation Annual precipitation Herbicide resistance	8.30e <sup>-3</sup> 5.73e <sup>-3</sup> 5.48e <sup>-3</sup> 3.57e <sup>-3</sup> 3.30e <sup>-3</sup> 2.34e <sup>-3</sup> 2.02e <sup>-3</sup> 1.67e <sup>-3</sup>

- 742 1. Mean of squared errors  
 743 2. Predictors are ordered by relative variable importance. Predictors also identified in  
 744 Ordinary Least Square regressions are in bold.  
 745 3. Measured by the decrease in model mean square error after removal of the corresponding  
 746 variable







