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Title: Shifts in outcrossing rates and changes to floral traits are associated with the evolution of herbicide resistance in the common morning glory

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Statement of authorship

AK collected seeds, performed experiments, analyzed data and wrote the paper; EF collected data; SMC collected data and contributed to the manuscript; RSB designed the study, performed the analyses, and wrote the paper. All authors discussed the results and commented on the manuscript.

Data accessibility

Upon acceptance, data will be made available through the Dryad public repository.

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Abstract

Human-mediated selection can strongly influence the evolutionary response of natural organisms within ecological timescales. But what traits allow for, or even facilitate, adaptation to the strong selection humans impose on natural systems? Using a combination of lab and greenhouse studies of 32 natural populations of the common agricultural weed, *Ipomoea purpurea*, we show that herbicide resistant populations self-fertilize more than susceptible populations. We also identify a potential mechanism to explain this higher rate of selfing in nature: highly resistant populations exhibit lower anther-stigma distance, a trait known to lead to high self-pollination in this species. Overall, our results extend the general finding that plant mating can be impacted by human-mediated agents of selection to that of the extreme selection of the agricultural system. This work highlights the influence of human-mediated selection on rapid responses of natural populations that can lead to unexpected long-term evolutionary consequences.

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Introduction

Pesticides are used world-wide to protect agricultural crops from the damaging effects of insects, fungi and weeds (Enserink *et al.* 2013) and are considered vital for maintaining the world's food supply (Lamberth *et al.* 2013). Recently we have begun to recognize that their use can negatively impact the reproduction and mating patterns of natural organisms such as bees (Williams *et al.* 2015), amphibians (Rohr & McCoy 2010), and plants (Pline *et al*. 2003; Thomas *et al*. 2004; Baucom *et al.* 2008; Londo *et al*. 2014) and can have long term evolutionary consequences on these non-target species. In the US, 40% of the pesticides applied across the 400 million acres of cropland are herbicides (*U S EPA* 2011), which impose extreme selection on naturally occurring agricultural weeds (Jasieniuk *et al.* 1996; Vigueira *et al*. 2013). Strikingly, while herbicide resistance has evolved in over 200 plant species worldwide (Heap 2014), it is largely unknown how the plant mating system may be impacted during, or influence, this process.

The mating system, or the relative rate of outcrossing versus selfing, impacts the effective population size (Nunney 1993), gene flow and the genetic diversity of populations (Hamrick & Godt 1996) and thus strongly influences the population biology of species. In plants, the mating system has been shown to be a labile trait (Barrett 1998; Karron *et al*. 2012) that can be altered by human impacts on natural ecosystems (Eckert *et al.* 2010). Habitat fragmentation by deforestation, for example, reduces the outcrossing rate of many forest tree species (Aguilar *et al.* 2006; Eckert *et al.* 2010). While the proximate factors underlying this effect are unknown, lower pollinator abundance and/or a reduction in the number of potential mates in the population are thought to be involved

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(i.e., demographic bottleneck) (*reviewed in* Eckert *et al.* 2010). Further, self-pollinating plants, in contrast to those that rely on mates, would be more likely to maintain fitness in low abundance populations (known as reproductive assurance; Baker 1955, 1974) and contribute to overall lower outcrossing rates in fragmented populations. Another robust example of mating system change following human-mediated impacts comes from plant populations adapted to heavy-metal contaminated soils. Surveys of a number of plant species show that metal-adapted populations exhibit higher rates of autonomous self-pollination compared to conspecific non-adapted biotypes (Antonovics *et al.* 1971; Levin 2010), strongly suggesting selfing as a mechanism that reduces or prevents the influx of non-adapted, susceptible alleles into metal tolerant parental lines (Antonovics 1968). These examples clearly illustrate that mating systems can be influenced by or concomitantly evolve in response to human imposed selection in natural systems.

Herbicides are strong agents of selection as they are designed to remove 90% of a weedy plant population (Jasieniuk *et al.* 1996). Because their use can lead to demographic bottlenecks and subsequent rapid adaptation, the effects of herbicide on population dynamics are analogous to the above scenarios of forest fragmentation and metal-contaminated soils. While we know that low doses of herbicide can reduce pollen production of plants (Baucom *et al.* 2008; Londo *et al.* 2014), we do not know how mating systems are influenced when plant populations are consistently exposed to herbicide application and subsequently evolve herbicide resistance. We hypothesize that the mating system of an agricultural weed should co-vary with the level of herbicide resistance in nature. Resistant individuals which survive herbicide application likely face a mate-limited environment, giving self-pollinating individuals a reproductive advantage

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over ones that rely on cross-pollination (i.e., reproductive assurance; Baker 1955, 1974; Goodwillie *et al.* 2005; Pannell *et al.* 2015). Further, the self-pollination of resistant types would reduce the influx of non-adapted, susceptible alleles and thus effectively maintain resistance within their progeny (Antonovics 1968). Both reproductive assurance and the prevention of gene flow hypotheses predict an association between herbicide resistance and higher selfing rates. Interestingly, while many herbicide resistant weed species are reported to be predominantly selfing (Jasieniuk *et al.* 1996), there are no investigations, to our knowledge, that examine the potential for co-variation between the mating system and herbicide resistance in nature, a finding that would indicate a role for the mating system when populations respond to the strong selection imparted by herbicides.

Ipomoea purpurea, an annual weed of agricultural fields and disturbed sites in the southeastern and Midwest US is a model for examining persistence in stressful and competitive environments (Baucom *et al.* 2011; Chaney & Baucom 2014). As such the species is a particularly relevant candidate for examining how mating systems may be influenced by human-mediated selection. Populations of this agricultural weed have been exposed consistently to the application of glyphosate, the active ingredient in the herbicide RoundUp, since the late 1990's given the wide spread adoption of RoundUp Ready crops in the US (NASS 2015). Recent work shows the existence of variation among populations for the level of resistance to glyphosate across its North American range; while some populations of *I. purpurea* exhibit 100% survival following application of the field-dose of the herbicide, other populations exhibit high susceptibility (Fig. 1a) (Kuester *et al.* 2015). In addition, natural populations of this hermaphroditic species exhibit an average outcrossing rate of 65-70%, indicating that it employs a mixed

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mating strategy (Ennos 1981; Brown & Clegg 1984). Empirical work has also shown that *I. purpurea* plants with a smaller anther-stigma distance (ASD, the distance between the tallest anther and the stigma; Fig. 1b) self-pollinate more often than individuals with a larger ASD (Ennos 1981; Chang & Rausher 1999a). Because both anther-stigma distance and glyphosate resistance in this species are heritable and respond to selection (Chang & Rausher 1999b; Debban *et al*. 2015), this common agricultural weed species provides a unique opportunity to examine the potential that mating systems and associated reproductive traits evolve in response to extreme regimes of selection imposed by herbicide.

Here, we determine if the mating system of *Ipomoea purpurea* co-varies with herbicide resistance, and if reproductive traits associated with self-pollination are likewise influenced by resistance status. We previously estimated the percent survival of populations following herbicide application using a replicated dose-response greenhouse experiment with individuals sampled as seed from the field in 2012. We used these population-level estimates of survival as each population's resistance level (Fig. 1a) (Kuester *et al.* 2015), and determined if molecular-marker based estimates of the mating system and measurements of floral morphology co-varied with the level of herbicide resistance in natural populations. Further, our experimental populations were sampled twice, once in 2003 and again in 2012 from the same location (Fig. 1a), allowing us to examine the hypothesis that floral traits have changed in these populations over time. We predict that populations with a high level of glyphosate resistance should exhibit evidence of reduced outcrossing and reductions in the anther-stigma distance – either of which

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would indicate that plant reproductive traits exhibit correlated evolution in response to strong selection from herbicide application.

Materials and Methods

Mating system estimates. We performed a progeny array analysis to estimate the mating system of 24 populations located in the southeastern and Midwestern US (indicated by triangles in Fig. 1a). These populations are part of a previous study in which we screened for glyphosate resistance, defined as the proportion each population that survived the application of 1.7 kg a.i./ha RoundUp (Kuester *et al.* 2015), a rate that is slightly higher than the current recommended field dose. One seed randomly selected from an average of 11 fruits per maternal line from an average of 19 maternal lines per population were used to estimate mating system parameters (see Appendix S1 in Supporting Information for numbers of maternal lines and progeny per population). Maternal plants were sampled at least two meters apart within each agricultural field. DNA was extracted from seedling cotyledon tissues using a CTAB method (T Culley, pers. comm.). In total, 4798 progeny were genotyped with fifteen previously described microsatellite loci for maternal-line estimates of the mating system (see Kuester *et al.* 2015 for specific details of the PCR conditions). All sample genotypes were analyzed using Applied Biosystems PeakScanner 1.0 analytical software (Carlsbad, CA) with a PP (Primer Peaks adjustment) sizing default, and scoring was double-checked manually for errors in a random sub-sample of 200 individuals. We examined our ability to assign parentage using Cervus (Kalinowski *et al.* 2007) and determined population-level estimates of genetic diversity and inbreeding using GenalEx (Peakall & Smouse 2012). We estimated mating system parameters using

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BORICE (Koelling *et al.* 2012), which is a Bayesian method to estimate the family-level outcrossing rate (t) and maternal line inbreeding coefficients (F) (Koelling *et al.* 2012). BORICE is reported to perform well when either family sizes are small or maternal genotypes are unavailable (Koelling *et al.* 2012). We used the following default parameters when estimating mating system parameters: 1 million iterations and 99,999 burn-in steps, an outcrossing rate tuning parameter of 0.05, an allele frequency tuning parameter of 0.1, and an initial population outcrossing rate of 0.5. We examined the possibility that null alleles influenced our mating system estimates by re-running all analyses after excluding 4 loci that potentially exhibited null alleles (i.e., loci with ~25% null alleles: IP18, IP1, IP26 and IP42) as indicated by MicroChecker (Van Oosterhout et al. 2004). We found no evidence that null alleles impacted estimates across populations (correlation between outcrossing rates for all loci and 4 loci removed: $r = 0.94$, $P < 0.001$) and thus report results using all 15 loci.

Floral phenotypes. We performed a replicated greenhouse experiment to determine if floral morphology varied according to resistance level and to determine if floral traits differed between two sampling years (2003 and 2012). Seeds were sampled from maternal plants from 32 randomly chosen populations located in the Southeast and Midwest US in the fall of 2012 (all populations in Fig. 1a); sixteen of these populations had been previously sampled in 2003 (see Appendix S2). We planted seeds from between 1-29 maternal lines (average=12.74, median=13; see Appendix S2 for number of individuals) from each population and each sampling year in 4-inch pots in a completely randomized design at the Matthaei Botanical Gardens at the University of Michigan (Ann

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Arbor, MI). To increase overall sample size, a second replicate experiment was started two weeks later in the greenhouses for a total of 640 experimental plants. Once plants began flowering, we measured the height of the pistil (cm) and the tallest stamen (cm) to estimate anther-stigma distance (ASD) of an average of 5.5 flowers per plant across 17 sampling dates. An average of 2 flowers were measured from each plant each sampling date; measurements taken on multiple flowers per plant per sampling date were averaged prior to analysis. Over the course of the experiment, we measured 3569 flowers from 622 experimental plants.

Statistical analyses. To determine if mating system parameters (the outcrossing rate (t), maternal inbreeding coefficient (F)) of *I. purpurea* co-varied with resistance, we performed partial linear regressions using the lm function in R v 3.1.1 (Team 2013) in which each population's mating system estimate was used as the dependent variable with the level of resistance (proportion survival at 1.7 kg a.i./ha) along with the latitude included as independent variables. Each parameter was examined for normality by performing a Shapiro-Wilk test and was transformed if necessary to meet the assumptions of linear regression.

We examined the potential that floral morphology differed according to the level of resistance first using populations sampled in 2012 (N = 32). To do so we performed a multivariate ANOVA with log-transformed values of each floral phenotype (anther-stigma distance, length of the tallest stamen, and pistil height) as dependent variables in the model and the experimental replicate, resistance level of the population, latitude of

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the population, sampling date, and the interaction between year and resistance level as fixed, independent variables in the model. Prior to analysis, we binned populations according to 2012 resistance level using kmeans clustering in R with a cluster value of 3 (R Core Team 2013). This strategy identified natural breaks in resistance values among populations and binned them as follows: 18 populations, 0-35% survival (Low); 15 populations, >35%-67% survival (Medium); and 3 populations, >67-100% survival (High) at 1.7 kg ai/ha of RoundUp.

We next ran separate univariate analyses for each trait to determine if floral traits differed according to resistance level and if they have changed over sampling years. For each model, the log-transformed floral trait of interest (ASD, height of tallest anther, pistil height) was the dependent variable with the experimental replicate, resistance level, sampling date, and latitude as independent variables in the models. Analyses indicated that each floral trait varied significantly according to resistance level, and thus a series of non-orthogonal comparisons of floral traits between resistance levels (H vs L, H vs M, M vs L) were performed using Wilcoxon nonparametric t-tests followed by p-value adjustments using the Holm method of the p.adjust function in R v 3.1.1 (R Core Team 2013). To determine if floral morphology had changed between sampling years and/or if floral morphology differed between years differently for the high, medium, and low resistance populations, we performed the same analyses as above using only populations that were sampled in both 2003 and 2012 (N = 15), including a year term and a year by resistance level term in each analysis.

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Literature review of resistant plant mating system. We performed literature searches for mating system descriptions (selfing, mixed mating, or outcrossing) of herbicide resistant species in the United States (N = 69; from http://weedscience.org/). Species were considered self-fertilizing if the species was described as "highly" or "predominantly" (>90%) selfing and considered as outcrossing if described in the literature as "highly" or "predominantly" outcrossing. Species were considered to exhibit mixed mating if they were described as explicitly such or as capable of producing seed through both selfing and outcrossing. Of the 69 herbicide resistant species, we were able to identify the mating system for 66 species. For comparison, we randomly selected species from a list of 'weedy' US species from the Composite List of Weeds compiled by the Standardized Plant Names subcommittee and published on Weed Science Society of America (WSSA) website (www.wssa.net) and likewise performed literature searches for a description of their mating system. We searched the literature until we found descriptions for 66 weedy, non-resistant species, which required searching the literature for 194 species. We used a Fisher's exact test to compare the proportion selfing versus any outcrossing (i.e., species described as mixed mating or outcrossing) between herbicide resistant and non-resistant weeds.

Results

Mating system. The average combined exclusion probabilities across all populations was high, at greater than 99%, indicating that the fifteen microsatellite loci successfully assigned parentage (see Appendix S1). Values of the outcrossing rate varied substantially

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across populations (range: 0.27-0.8), with an average value (±SE) for the species of 0.50 (±0.03) (Appendix S3).

We uncovered a strong and striking negative relationship between the average family-level outcrossing rate of each population (Appendix S3) and the level of glyphosate resistance (Fig. 2a; Table 1); individuals from highly resistant populations tend to self-pollinate more than individuals from less resistant populations. An analysis of variance identified a significant effect of resistance on outcrossing rate ($P = 0.004$; Table 1), but no effect of the latitude of the population on the outcrossing rate ($P = 0.33$). We likewise found increased maternal inbreeding coefficients in highly resistant populations compared to susceptible populations (Fig. 2b, $P = 0.02$; Table 1). Similar to the multilocus outcrossing rate, we found no evidence that the maternal inbreeding coefficients were influenced by latitude (Table 1). Together, these results demonstrate that outcrossing rates were lower and maternal inbreeding coefficients higher in glyphosate resistant compared to susceptible populations, even after accounting for the effects of population location (*i.e.* latitude).

Floral morphology. A multivariate analysis of variance indicated that floral morphology likely related to selfing rates (anther-stigma distance (ASD), height of the pistil and the tallest stamen) was influenced by both the resistance level and sampling date (resistance level, $P < 0.005$; sampling date, $P < 0.001$; Appendix S4). In separate univariate ANOVAs, we found that ASD and stamen height differed according to the level of resistance (resistance level: ASD, $P = 0.002$; stamen height, $P = 0.003$, Appendix S5) and

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the sampling date (Appendix S5). Plants from the most resistant populations exhibited significantly smaller ASD than plants from the low- and medium-level resistance group (ASD, mean ± SE, high resistance: 0.49 ± 0.22 mm, medium: 1.29 ± 0.13 mm, low: 1.07 ± 0.10 mm; Fig. 3a). The lower ASD values of resistant plants are due to differences in the relative height of the tallest stamen, with stamen height of the most resistant populations ~0.8 mm taller than that of the low resistant populations (Fig. 3b). The pistil height exhibited no differences according to resistance level across all 32 populations (Fig. 3c).

There was no evidence that ASD either increased or decreased across collection years among populations (Year effect in ANOVA: $F_{1,1070} = 0.016$, P = 0.89; Fig. 3d). Despite the relative stability of ASD values among populations across this nine-year period, both floral traits comprising ASD – height of the tallest stamen and pistil height – showed a significant collection year by resistance level interaction (Stamen height: $F_{2, 1070} = 9.03$, $P < 0.001$; Pistil height: $F_{2, 1070} = 5.56$, $P < 0.01$, Fig. 3e-f). The stamen and pistil height both increased in the most resistant populations (Fig. 3e), but decreased in the medium resistance populations and remained the same in the low-resistance populations (Fig. 3e-f). Overall, our results show that ASD is lower in the most resistant populations compared to less resistant populations, and that the small ASD of the resistant populations remains stable across time, even in light of changes in the traits comprising ASD. These results provide evidence for a possible evolutionary mechanism underlying the lower outcrossing in resistant populations compared to the less resistant populations.

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Literature survey. To examine the generality of our findings, we collated published descriptions of the mating system of 66 of the 69 herbicide resistant US weed species and find that many (~53%) are described as predominantly selfing (Appendix S6), confirming earlier reports (Jasieniuk *et al.* 1996). In comparison, non-herbicide resistant weeds are more likely to exhibit mixed-mating or outcrossing relative to herbicide resistant weeds, as only 23% of non-resistant weeds are predominantly self-pollinating. The difference in the proportion of predominantly selfing versus outcrossing between resistant and non-resistant weed species was highly significant (Fisher's exact test, predominantly selfing vs any outcrossing $P < 0.001$). This macroevolutionary pattern thus supports the idea that herbicide resistance status is influenced by the mating system, *i.e.*, perhaps resistance alleles are more likely to be maintained in a population of a selfing species.

Discussion

In line with our predictions, we demonstrate that outcrossing rates are lower in herbicide resistant populations of the agricultural weed, *Ipomoea purpurea* compared to susceptible populations. We likewise find evidence that the most resistant populations exhibit lower anther-stigma distance compared to susceptible populations, indicating that low ASD is an evolutionary mechanism responsible for the increased rate of self-pollination in resistant populations. Further, the low ASD of highly resistant populations was maintained between sampling years, even in light of changes to the two traits comprising ASD. In addition, our survey of the literature indicates that herbicide resistant weeds are more likely to be highly self-pollinating than non-resistant weeds, suggesting that the findings from our within-species examination of the mating system in *I. purpurea*

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are analogous to broader, among-species patterns. Below, we discuss each of our major findings and place them in the broad context of mating system changes associated with human-mediated selection.

Plant mating and reproductive trait changes associated with resistance

As anticipated by both the reproductive assurance (Baker 1955, 1974; Goodwillie *et al.* 2005; Pannell *et al.* 2015) and 'prevention of gene flow' (Antonovics 1968) hypotheses, our work finds a significant negative relationship between the mating system and level of herbicide resistance across natural populations of an agricultural weed, indicating that individuals from resistant populations self more often than those from susceptible populations. There are currently few examinations of the mating system of xenobiotic tolerant or resistant species for comparison. In the most relevant example to date, metal-tolerant populations of the grass species *Anothoxanthum odoratum* and *Agrostis tenuis* exhibit higher self-fertility compared to nearby susceptible pasture populations (Antonovics 1968; Antonovics *et al.* 1971). Theoretical work by the same authors suggested that higher rates of selfing in the metal-tolerant populations should evolve to reduce the influx of non-adapted genotypes (i.e., the prevention of gene flow hypothesis) (Antonovics 1968); however, no potential mechanism was identified. Indeed, our result that herbicide resistant populations both outcross less in natural settings and exhibit, on average, a smaller anther-stigma distance compared to susceptible populations supports the prevention of gene flow hypothesis. Mechanisms that promote the self-pollination of adapted individuals, such as a lower anther-stigma distance, would reduce the level of gene flow from non-adapted individuals (Levin 2010), thus ensuring that the

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offspring produced in novel or stressful/marginal environments are likewise stress-tolerant (Levin 2010). In this way, the increased self-fertilization of adapted types is hypothesized to lead to reproductive isolation between adapted and non-adapted individuals (Antonovics 1968; McNeilly & Antonovics 1968), and potentially promote niche differentiation (Levin 2010). Such shifts toward a higher propensity to self-fertilize in marginal habitats may not be unusual in nature, as a higher propensity to self has been identified in metal tolerant populations of *Armeria maritime* (Lefebvre 1970) and *Thlaspi caerulescens* (Dubois *et al.* 2003) as well as serpentine tolerant *Mimulus* (Macnair & Gardner 1998). We emphasize, however, that the above cases compare the ability to produce seed autonomously in greenhouse conditions between adapted and non-adapted individuals, which does not always correlate with selfing rates in nature, whereas our work presents estimates of the outcrossing rate of *I. purpurea* sampled from natural conditions.

Our data are likewise consistent with the reproductive assurance hypothesis. Originally proposed by Baker (Baker 1955), this hypothesis predicts greater selfing ability in colonizing species since they are likely mate-limited when arriving to new areas. Reproductive assurance through self-fertility is broadly although not ubiquitously supported by empirical research in other plant systems, such as small or pollinator-limited populations of *Capsella* (Foxe *et al.* 2009; Guo *et al.* 2009), *Levanworthia* (Busch *et al.* 2011) and *Clarkia* (reviewed in Busch & Delph 2012). Agricultural weeds that experience selection from herbicide application or population reduction *via* other means, such as tilling, are analogous to species that colonize novel or new habitats. For example, assuming resistance alleles are initially at low frequency, individuals that survive

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herbicide application and re-colonize crop fields will then likely be mate limited. Those with a higher propensity to self-pollinate would be at a relative advantage compared to those that cannot self-pollinate. In support of reproductive assurance, we found that the resistant populations from our 2012 collections that show low ASD likewise exhibited high resistance in their 2003 sample (Survival post-herbicide: 2003, 95%; 2012, 91%). It is thus possible that resistant individuals more likely to self (low ASD types) in 2003 were at a reproductive advantage compared to resistant individuals less likely to self (high ASD). Regardless of whether the data reported herein are best explained by selection for reproductive isolation (Antonovics 1968) or the reproductive assurance hypothesis (*reviewed in* Pannell *et al.* 2015), the patterns we have uncovered among naturally-occurring populations of this common weed show that the plant mating system may play a significant role in the response to selection following the application of herbicides.

Recent multilocus simulations of the causes and consequences of selfing likewise support our empirical results. These simulations predict a shift in the mating system from primarily outcrossing to high rates of selfing in the presence of a moderate to highly beneficial allele (Kamran-Disfani & Agrawal 2014). The cause of this relationship is likely due to the segregation effect (Uyenoyama & Waller 1991): an allele that causes higher selfing enhances segregation between beneficial and resident alleles and forms associations with homozygotes. As selfing modifiers become associated with the beneficial mutation, these selfing individuals will respond quickly to selection and even higher rates of selfing would then evolve in the population (Kamran-Disfani & Agrawal 2014). This dynamic is proposed for species that are establishing a new habitat, or

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following episodes of environmental change (Kamran-Disfani & Agrawal 2014) – both of which are experienced by plants exposed to herbicide application.

As hypothesized above, the responses to selection and subsequent changes to the mating system that we have identified may be adaptive and due to a genetic basis, or could alternatively be due to plastic changes influenced by the environment (Rick *et al.* 1977; Brock & Weinig 2007; van Kleunen 2007; Vallejo-Marín & Barrett 2009). Several lines of evidence suggest that adaptation is the more likely cause for the patterns observed in this study. While we present field estimates of the mating system among populations, we measured floral morphology in a completely randomized greenhouse experiment using replicate maternal lines from many populations, with all individuals experiencing a common environment. Thus, the differences we report in ASD among populations cannot be explained by different environmental factors from different populations. Further, both anther-stigma distance and glyphosate resistance have an additive genetic basis in this species (Chang & Rausher 1998; Baucom & Mauricio 2008; Debban *et al.* 2015), both traits respond rapidly to artificial selection (Chang & Rausher 1998; Debban *et al.* 2015), and populations sampled for the work reported herein were all from agricultural fields with a history of glyphosate application. These reasons altogether suggest that the co-variation we have uncovered between floral phenotypes and resistance across many natural populations of this weed is likely due to adaptive changes following selection rather than plasticity in either the mating system or the level of ASD.

Our finding of a shift in the mating system to increased selfing and likewise a reduction in the level of ASD in the highly resistant populations is consistent with floral morphology changes associated with shifts to selfing in other self-compatible species.

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Increased selfing rates are commonly associated with three reproductive traits: decreased ASD, reduced time between the dehiscence of pollen and stigma receptivity (dichogamy) and reductions in flower size, often termed 'selfing syndrome'(Barrett *et al.* 2014). The decreased level of ASD in the highly resistant *Ipomoea* populations reported herein is a result of longer stamens rather than changes in the pistil height, a result similar to that found in selfing populations of the weedy plant *Eichhornia paniculata* (Vallejo-Marín & Barrett 2009). A change in ASD due to increased stamen length was the only component of the selfing syndrome that we identified. We found no evidence that flower size—either length or width of the corolla—was reduced in the highly resistant populations (*data not shown*), and we have yet to investigate the dichogamy among these experimental populations. Most species that show shifts to increased selfing—e.g., *Capsella*, *Collinsia*, and *Eichhornia*—exhibit one of these reproductive trait shifts rather than all three (Barrett *et al.* 2014), and thus our results are in line with that observed in shifts to selfing in other systems.

Broad patterns among herbicide resistant weeds

Although anecdotal reports suggest many herbicide resistant plants are predominantly selfing (Jasieniuk *et al.* 1996), ours is the first to identify co-variation between herbicide resistance and estimates of the outcrossing rate, thus providing empirical evidence that the mating or breeding system of a plant plays a role in the response to selection *via* herbicide. Our survey of the literature finds that the majority of herbicide resistant weeds are more likely to be described as 'predominantly selfing' in comparison to non-herbicide resistant weedy species. Such findings suggest that selfers

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may be more successful in maintaining resistance alleles since self-pollination will reduce the potential for crossing with non-resistant types. Although suggestive, there are caveats of this broad view of the literature. First, the association we identify could be due to phylogenetic constraints, as previous work finds that the majority of herbicide resistant agricultural weeds in the US belong to 9 plant families (Kuester *et al*. 2014). Second, we searched for and report descriptions of the mating system since explicit measures of the outcrossing rate for herbicide resistant species are unavailable in the literature. That our within-species examination of *I. purpurea* populations finds increased selfing as herbicide resistance concomitantly increases, however, provides a potential explanation for the high abundance of self-pollination among herbicide resistant plants. Future efforts examining the mating system as well as reproductive traits in other herbicide resistant species will help to determine the ubiquity of our findings.

Conclusions

Overall, we have demonstrated that individuals from herbicide resistant populations self more than those from susceptible populations in natural settings and that low anther-stigma distance may be a potential mechanism underlying this increased rate of selfing. These results suggest a critical role for the mating system in the evolution of herbicide resistance and provide a potential explanation for the high proportion of herbicide resistant species that are predominantly selfing. Further, our work identifies human impacts on plant mating patterns that go beyond the indirect consequences of environmental manipulations such as forest fragmentation and metal contamination. Changes in mating systems can have cascading effects on the effective population size

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(Nunney 1993), gene flow and the genetic diversity of natural populations (Hamrick & Godt 1996) and can determine the overall evolutionary propensity of these species. Our findings thus highlight the importance of considering the influence of human-mediated selection on correlated responses of natural populations that can lead to long-term evolutionary consequences. Likewise, these results show that associations between highly beneficial traits and plant reproduction can occur rapidly within ecological timescales. The results of our work are thus applicable to other scenarios of strong selection such as climate change or scenarios wherein individuals of colonized populations are mate-limited.

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Tables

Table 1. Results of separate linear regressions testing the influence of resistance and population location (latitude) on mating system parameters. Effects that are significant ($P < 0.05$) are bolded.

Effect		Outcrossing rate (t)		Maternal inbreeding coefficient (F)	
	Df	F	P	F	P
Resistance Level	1	10.693	**0.004**	6.022	**0.023**
Latitude	1	0.997	0.329	0.894	0.355
Residuals	21				

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Figures

Figure 1. Map of populations sampled within the USA, **a**, and image of anther-stigma distance in *I. purpurea*, **b**. Floral morphology was measured for all populations (N = 32) whereas the mating system was estimated for populations indicated by triangles (N = 24). The color indicates the resistance level for each population based on proportion survival following application of 1.7 kg ai/ha of herbicide, which is slightly more than the currently recommended field dose of herbicide (from Kuester *et al.* 2015). Sites were sampled at least 5 km apart.

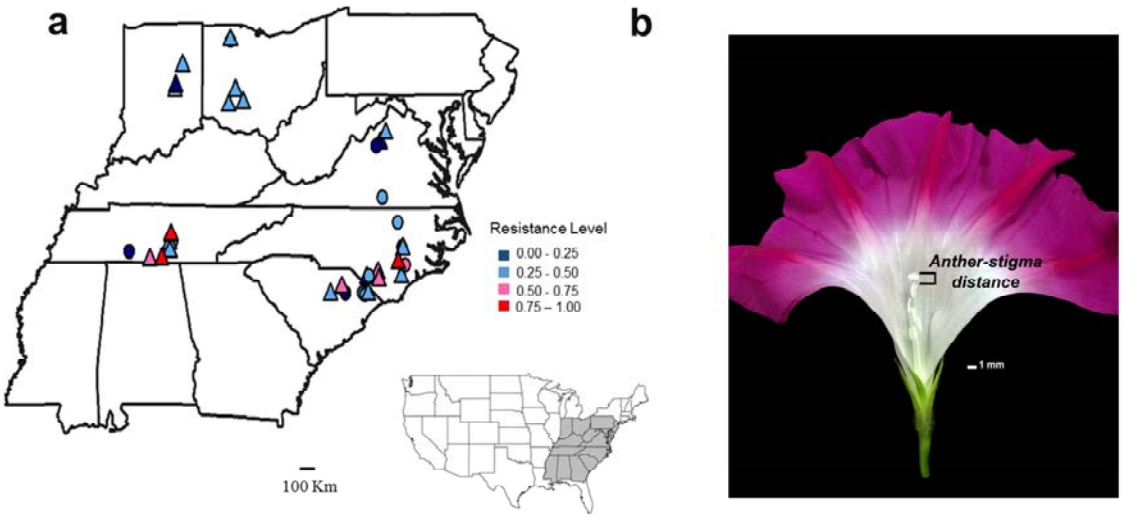

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Figure 2. The relationship between mating system parameters and the proportion survival of the population following application of 1.7 kg ai/ha of herbicide in *I. purpurea*. **a**, outcrossing rate (t), **b**, mean inbreeding coefficient of maternal individuals (F). Significant relationships are indicated by a regression line.

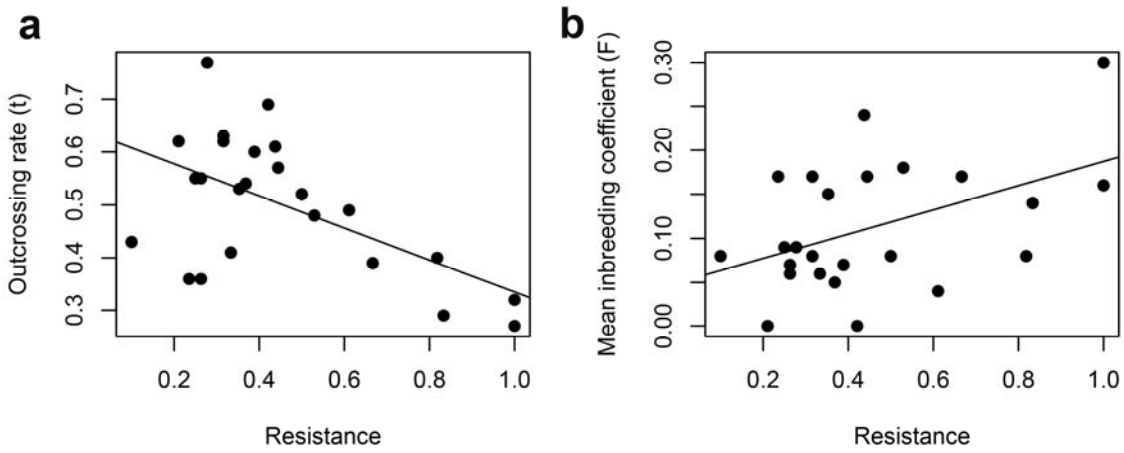

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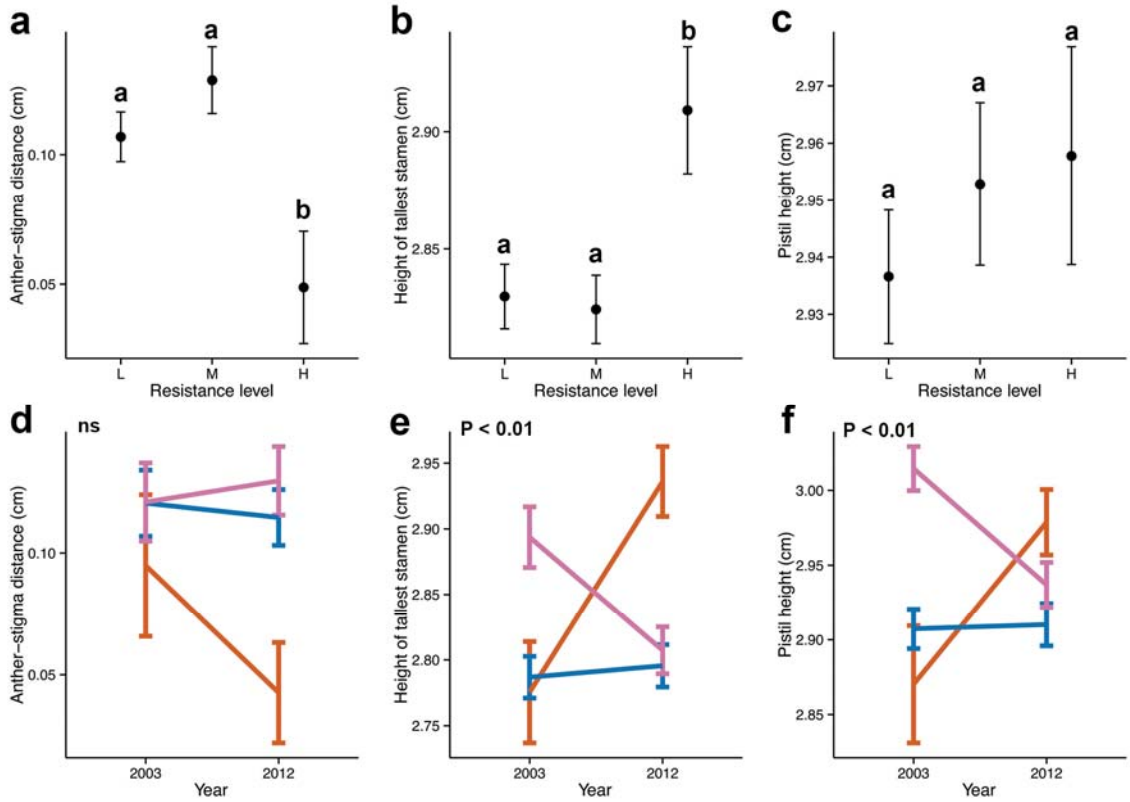
Figure 3. Anther-stigma distance varies according to resistance level but shows no change between sampling years. **a**, anther-stigma distance (cm), **b**, height of the tallest stamen (cm), **c**, pistil height (cm) averaged across both sampling years for the low-, medium, and high-resistance levels. Lower-case letters indicate if the resistance levels differed from one another, following corrections for multiple tests. **d**, anther-stigma distance, **e**, height of the tallest stamen (cm), and **f**, pistil height (cm) averaged per sampling year, with error bars indicating 1SE from the mean. The p-value indicates the significance of the interaction between sampling year and resistance level. The resistance levels are indicated as follows: Low, dark blue = 0.00 – 0.35; Medium, pink >0.35 – 0.67; High, red >0.67 – 1.00 survival following 1.7 kg ai/ha of RoundUp.



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Appendix

Table S1. The number of maternal lines and progeny used to estimate mating system parameters along with estimates of genetic diversity (expected and observed heterozygosity), polymorphic information content, and probabilities of exclusion of 24 *I. purpurea* populations at 15 polymorphic loci.

Table S2. The number of individual plants used per population in the greenhouse floral phenotyping experiments. Shown is the total number of individual plants summed across two experimental replicates for each year that seeds were sampled from natural populations.

Table S3. Mating system parameters estimated using BORICE.

Table S4. Results of a MANOVA testing the influence of the population resistance level, floral trait sampling date, population latitude on multivariate phenotype of anther-stigma distance (cm), stamen (cm) and pistil height (cm) using all 32 populations sampled in 2012.

Table S5. Results of separate ANOVAs testing the influence of population resistance level, floral trait sampling date, and population latitude on anther-stigma distance (cm), pistil height (cm) and height of tallest stamen (cm) separately.

Table S6. The proportion of herbicide resistant weeds and a random sample of non-resistant weeds that are described as selfing, mixed mating, or outcrossing from a database of US weeds.

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Table S1. The number of maternal lines and progeny used to estimate mating system parameters along with estimates of genetic diversity (expected and observed heterozygosity), polymorphic information content, and probabilities of exclusion of 24 *I. purpurea* populations at 15 polymorphic loci.

POPID	Pop	Nmats	Nind	Nloci	Na	PIC	PEP	PESP	PEC	PEI	PESI	Ho	He	FIS	sig
1	BI	13	70	15	2.80	0.238	0.540	0.879	0.975	1.000	0.986	0.15 (0.06)	0.18 (0.05)	0.206	
2	BU	17	172	15	3.87	0.340	0.760	0.965	0.997	1.000	0.998	0.22 (0.06)	0.29 (0.05)	0.251	*
4	CR	5	47	15	2.53	0.255	0.661	0.899	0.979	1.000	0.992	0.27 (0.08)	0.30 (0.05)	0.105	
8	DA	21	240	15	3.20	0.252	0.611	0.898	0.981	1.000	0.991	0.23 (0.09)	0.25 (0.05)	0.082	
10	DW	30	292	15	3.80	0.400	0.857	0.982	0.999	1.000	1.000	0.15 (0.04)	0.21 (0.05)	0.321	*
15	HO	21	275	15	4.00	0.378	0.819	0.977	0.999	1.000	0.999	0.27 (0.06)	0.36 (0.04)	0.255	*
19	MK	9	85	15	3.47	0.361	0.806	0.969	0.997	1.000	0.999	0.23 (0.06)	0.29 (0.05)	0.216	
21	NH	7	29	15	2.80	0.346	0.791	0.964	0.996	1.000	0.999	0.31 (0.08)	0.42 (0.04)	0.251	
23	SN	20	200	15	3.47	0.387	0.848	0.982	0.999	1.000	0.999	0.25 (0.07)	0.31 (0.05)	0.191	
26	SP	22	244	15	4.20	0.465	0.930	0.994	1.000	1.000	1.000	0.28 (0.08)	0.32 (0.05)	0.127	
28	SU	21	308	15	5.53	0.499	0.943	0.997	1.000	1.000	1.000	0.28 (0.07)	0.36 (0.05)	0.222	
29	TA	25	325	15	3.93	0.477	0.925	0.995	1.000	1.000	1.000	0.3 (0.07)	0.44 (0.04)	0.314	*
30	WI	20	417	15	4.60	0.488	0.938	0.996	1.000	1.000	1.000	0.22 (0.08)	0.28 (0.06)	0.224	
32	WG	26	227	15	3.40	0.456	0.923	0.992	1.000	1.000	1.000	0.25 (0.07)	0.31 (0.05)	0.216	
33	SCOH	20	223	15	3.73	0.353	0.797	0.967	0.997	1.000	0.999	0.32 (0.08)	0.29 (0.05)	-0.09	
34	OH5	18	182	15	3.27	0.379	0.844	0.980	0.999	1.000	0.999	0.25 (0.07)	0.24 (0.04)	-0.02	
35	IN9	20	210	15	3.27	0.335	0.787	0.964	0.996	1.000	0.998	0.2 (0.07)	0.20 (0.05)	0.019	
36	IN12	20	207	15	3.93	0.374	0.816	0.975	0.998	1.000	0.999	0.23 (0.08)	0.25 (0.05)	0.071	
38	OH3	18	150	15	3.53	0.381	0.824	0.976	0.998	1.000	0.999	0.2 (0.05)	0.25 (0.05)	0.205	
39	IN10	15	116	15	3.27	0.246	0.541	0.891	0.980	1.000	0.988	0.26 (0.07)	0.32 (0.05)	0.186	
40	COH	20	224	15	3.53	0.277	0.664	0.925	0.989	1.000	0.994	0.25 (0.07)	0.26 (0.05)	0.042	
41	SH5	20	198	15	3.40	0.392	0.853	0.981	0.999	1.000	0.999	0.23 (0.05)	0.36 (0.05)	0.363	*
42	SH4	16	143	15	3.53	0.437	0.901	0.990	1.000	1.000	1.000	0.24 (0.07)	0.29 (0.05)	0.165	
43	VA2	21	214	15	3.73	0.446	0.900	0.991	1.000	1.000	1.000	0.26 (0.07)	0.41 (0.04)	0.372	*

Nmat = Number of maternal lines per population; Nind = Number of individuals genotyped; Na = Average number of alleles per locus, PIC = polymorphic information content; PESP = probability of exclusion of a single parent; PEP = exclusion for paternity (mother known); PEC = exclusion probability of a parent pair; PESI = exclusion probability of identity across outcrossed sibs. He = expected heterozygosity, Ho = observed heterozygosity; Inbreeding coefficients (FIS) and significance value estimated using FSTAT (Goudet 1995). *P-value < 0.05, indicating inbreeding coefficients that are significantly greater than 0.

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Table S2. The number of individual plants used per population in the greenhouse floral phenotyping experiments. Shown is the total number of individual plants summed across two experimental replicates for each year that seeds were sampled from natural populations.

POPID	Number of maternal individuals, 2012	Number of maternal individuals, 2003
1	22	10
2	15	13
4	17	16
8	12	16
9	19	-
10	18	-
12	13	18
14	18	16
15	12	15
16	29	13
17	18	15
19	20	17
21	8	-
22	27	18
23	8	-
25	1	17
26	6	-
28	13	16
29	10	14
30	9	-
32	10	6
34	7	-
35	7	-
36	8	-
38	3	-
39	7	-
40	9	-
41	8	-
42	10	-
44	8	-
45	6	-
52	1	-

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Table S3. Mating system parameters estimated using BORICE.

POPID	Pop	Nmats	Nloci	t, BORICE (SE)	F, BORICE (SE)
1	BI	13	15	0.27 (0.005)	0.16 (0.003)
2	BU	17	15	0.53 (0.003)	0.15 (0.004)
4	CR	5	15	0.62 (0.006)	0 (0.001)
8	DA	21	15	0.48 (0.004)	0.18 (0.003)
10	DW	30	15	0.32 (0.002)	0.30 (0.003)
15	HO	21	15	0.55 (0.004)	0.07 (0.002)
19	MK	9	15	0.39 (0.004)	0.17 (0.005)
21	NH	7	15	0.61 (0.006)	0.24 (0.006)
23	SN	20	15	0.52 (0.004)	0.08 (0.003)
26	SP	22	15	0.54 (0.003)	0.05 (0.003)
28	SU	21	15	0.62 (0.002)	0.08 (0.001)
29	TA	25	15	0.49 (0.002)	0.04 (0.002)
30	WI	20	15	0.69 (0.002)	0 (0.001)
32	WG	26	15	0.29 (0.006)	0.14 (0.003)
33	SCOH	20	15	0.41 (0.002)	0.06 (0.002)
34	OH5	18	15	0.77 (0.010)	0.09 (0.003)
35	IN9	20	15	0.36 (0.003)	0.06 (0.003)
36	IN12	20	15	0.55 (0.002)	0.09 (0.003)
38	OH3	18	15	0.57 (0.004)	0.17 (0.004)
39	IN10	15	15	0.36 (0.006)	0.17 (0.005)
40	COH	20	15	0.63 (0.006)	0.17 (0.004)
41	SH5	20	15	0.60 (0.005)	0.07 (0.003)
42	SH4	16	15	0.43 (0.003)	0.08 (0.003)
43	VA2	21	15	0.40 (0.004)	0.08 (0.002)

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Table S4. Results of a MANOVA testing the influence of the population resistance level, floral trait sampling date, population latitude on multivariate phenotype of anther-stigma distance (cm), stamen (cm) and pistil height (cm) using all 32 populations sampled in 2012.

	Df	Pillai	approx F	num DF	den DF	P
Replicate	1	0.005	1.548	3	861	0.201
Resistance level	2	0.021	3.087	6	1724	**<0.005**
Date	17	0.521	10.669	51	2589	**<0.001**
Latitude	1	0.007	2.135	3	861	0.094
Residuals	863					

Table S5. Results of separate ANOVAs testing the influence of population resistance level, floral trait sampling date, and population latitude on anther-stigma distance (cm), pistil height (cm) and height of tallest stamen (cm) separately.

	Anther-stigma distance (cm)			Tallest stamen height (cm)			Pistil height (cm)		
Effect	Df	F	P	Df	F	P	Df	F	P
Replicate	1	2.922	0.088	1	0.115	0.735	1	2.796	0.095
Resistance level	2	6.039	**0.002**	2	5.758	**0.003**	2	1.182	0.307
Date	17	7.418	**<0.001**	17	22.938	**<0.001**	17	21.255	**<0.001**
Latitude	1	0.008	0.929	1	5.032	**0.025**	1	5.009	**0.025**
Residuals	863			863			863		

Table S6. The proportion of herbicide resistant weeds and a random sample of non-resistant weeds that are described as selfing, mixed mating, or outcrossing from a database of US weeds.

Reported mating system	Non-resistant weeds	Resistant weeds
Selfing	0.227	0.530
Mixed mating	0.288	0.136
Outcrossing	0.485	0.333