Title: A resurrection experiment finds evidence of both reduced genetic diversity and potential adaptive evolution in the agricultural weed *Ipomoea purpurea*

Short title: Adaptive evolution in an agricultural weed

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Abstract

Despite the negative economic and ecological impact of weeds, relatively little is known about the evolutionary mechanisms that influence their persistence in agricultural fields. Here, we use a resurrection ecology approach and compare the genetic and phenotypic divergence of temporally sampled seed progenies of *Ipomoea purpurea,* an agricultural weed that is resistant to glyphosate, the most widely used herbicide in current-day agriculture. We found striking reductions in allelic diversity between cohorts sampled nine years apart (2003 vs 2012), suggesting that populations of this species sampled from agricultural fields have experienced genetic bottleneck events that have led to lower neutral genetic diversity. Heterozygosity excess tests indicate that this bottleneck may have occurred prior to 2003. Further, a greenhouse assay of individuals sampled from the field as seed found that populations of this species, on average, exhibited modest increases in herbicide resistance over time. Our results show that populations of this noxious weed, capable of adapting to strong selection imparted by herbicide application, may lose genetic variation as a result of this or other environmental factors. We likely uncovered only modest increases in resistance between sampling cohorts due to a strong and previously identified fitness cost of resistance in this species, along with the potential that non-resistant migrants germinate from the seed bank.

Introduction

The influence of human mediated selection is perhaps nowhere more prevalent than in the agricultural system. Agricultural weeds, in particular, provide excellent case studies of adaptation to human-mediated selection (Baker 1974). They are exposed to fertilizers, herbicides, irrigation, as well as variable cropping techniques, and these manipulations can impose frequent, strong, and highly predictable disturbance regimes (Barrett 1988). Examples of rapid adaptation to these scenarios are present in the literature from early cases of crop mimicry (Baker 1974; Barrett 1983; Barrett 1988) to the many recent examples of the evolution of herbicide resistance (Heap 2016). Although weeds are in many cases models for understanding rapid evolution and persistence in stressful environments, we currently have a limited understanding of the broad genetic changes that may influence weed populations in agricultural landscapes (Vigueira *et al.* 2013; Waselkov & Olsen 2014). These lapses in our knowledge are striking because the population dynamics of agricultural weeds are directly relevant to the global food supply. Agricultural weed infestations reduce world-wide crop yield by as much as 10% (Oerke 2005) and it has been estimated that crop losses caused by weeds cost the US agricultural economy ~33B USD per year (Pimentel *et al.* 2005). Clarifying the evolutionary forces that impact agricultural weeds can provide information on the process of rapid evolution more broadly as well as insight on how weeds survive and persist in agricultural regimes.

Although agricultural weeds co-exist and compete with crops, they evolve though unintentional human mediated selection rather than through direct artificial selection (Stewart & Warwick 2005) and represent a novel evolutionary state that is "neither wild nor domesticated"

74 (Vigueira *et al.* 2013). Weeds are subject to the same forces influencing evolution in nature—
75 notably, genetic drift, selection, and gene flow (Jasieniuk *et al.* 1996)—but they often experience
76 a selection intensity that is much higher than what is usually found in other natural systems. For
77 example, the predominant form of weed control in current farming is through the use of
78 herbicides, which are designed to remove 90% of the weed population (Jasieniuk *et al.* 1996;
79 Délye *et al.* 2013). Individuals that survive this high intensity of selection due to either chance or
80 genetic predisposition are founders for the next generation. Since the point of weedy plant
81 control regimes—whether through the use of herbicide or another control technique—is to
82 remove of a large portion of the population, populations that re-colonize are hypothesized to
83 show a pattern of constriction and genetic bottleneck (Jasieniuk *et al.* 1996; Vigueira *et al.*
84 2013). As a result, weeds could lose rare alleles important to future adaptation (Nei *et al.* 1975).

85 In support of this idea, population genetic surveys have found that weeds tend to exhibit
86 less genetic variation than other groups of plants (Hamrick *et al.* 1979), and there is some
87 evidence that weed populations from cultivated land exhibit decreased neutral genetic diversity
88 compared to wild populations (Kane & Rieseberg 2008). The majority of the work to date,
89 however, has compared populations across space, *i.e.,* from cultivated and non-cultivated areas
90 (Muller *et al.* 2010), or "wild" versus "weedy" populations (Kane & Rieseberg 2008). In
91 contrast, a novel approach that can provide direct evidence for evolutionary change through time
92 is by the use of "resurrection ecology" in which ancestor and descendant strains of species are
93 compared. In this type of experiment, seeds or propagules sampled from an earlier time point are
94 germinated after remaining dormant for a number of years and compared to descendant
95 populations sampled from the same location (Franks *et al.* 2007; Orsini *et al.* 2013). Although

96 resurrection ecology experiments have been used to address key questions on evolutionary
97 constraints in microbial systems (Lenski & Travisano 1994; Lenski 1998), such experiments in
98 eukaryotes have thus far used either a limited number of accessions (Baucom & Mauricio 2010)
99 or a limited number of distinct populations (Franks *et al.* 2007; Thomann *et al.* 2015).

100 Here we perform a resurrection ecology experiment to examine the population genetics
101 and potential adaptability of *Ipomoea purpurea*, the common morning glory. This species is
102 native to the central highlands of Mexico (Clegg & Durbin 2000; Defelice 2001) and is an
103 introduced invader of agricultural and disturbed areas in the United States (Defelice 2001).
104 Although the precise history of introduction into the US is unknown (Fang *et al.* 2013), lineages
105 in the US exhibit low diversity relative to Mexican accessions, suggesting a severe bottleneck
106 occurred following introduction (Fang *et al.* 2013). *I. purpurea* is considered one of the worst
107 weeds of southeastern US agriculture (Baucom *et al.* 2011), in part because it exhibits low-level
108 resistance to glyphosate, the most utilized herbicide in agriculture world-wide (Powles 2008).
109 Recently, we have identified a mosaic of glyphosate resistance across the US, with some
110 populations of *I. purpurea* exhibiting high resistance (a high proportion of the population that
111 survives glyphosate) and others showing high susceptibility post-herbicide application (Kuester
112 *et al.* 2015). Previous work has also found that an additive genetic basis underlies glyphosate
113 resistance in this species (Baucom & Mauricio 2008) and that resistance segregates in genetic
114 lines developed from a single population (Debban *et al.* 2015). Although *I. purpurea* populations
115 are found primarily within agricultural fields that are treated with glyphosate and other
116 herbicides, the impact of such strong selection, and any associated environmental changes on the
117 population genetics of this species over time remains largely unknown. Because seeds remain

viable for many years in lab conditions, we are able to examine both neutral and adaptive genetic variation of populations sampled from the same agricultural fields at two different time points—once in 2003 and again in 2012 (see Figure 1 for population locations). We first determine if the neutral genetic differentiation and diversity of *I. purpurea* populations have changed between sampling years. We pair this with greenhouse experiments to examine the potential that these populations, sampled from the same fields that were used for either soy or corn farms between 2003 and 2012 (Table S1) exhibit increased resistance over time. We find evidence of both genetic bottleneck and slight increase in the level of resistance, indicating that a noxious weed potentially adapts to the extreme selection imposed by herbicide applications even as genetic diversity decreases. We further find some indication that highly resistant populations exhibit lower genetic diversity than less resistant populations, suggesting that herbicide application is responsible for the reduction in neutral genetic diversity. This is the first examination, to our knowledge, of a resurrection ecology experiment that simultaneously identifies both loss of genetic diversity of an agricultural weed over time as well as potential evidence for adaptive evolution.

Materials and Methods

Population sampling. Locations and sampling strategies for 44 *I. purpurea* populations were previously described in Kuester *et al.* (2015). Twenty-six of these populations were sampled in 2003 and resampled in 2012 (see Figure 1 and Table S1). In 2003, we collected replicate seeds from between 6-30 maternal individuals at least 1 m apart from one another along a linear transect. We located the same populations in the fall of 2012 using GPS coordinates, which are

140 accurate to within a few meters. Agricultural fields are highly disturbed by tilling and harvesting
141 each year, and morning glories are predominantly found in areas that have recently experienced
142 soil disturbance *via* tilling; as a result, this system is not amenable to the maintenance of long-
143 term transects. We are thus making the assumption that adult plants present within the same
144 agricultural field, and located within the nearest distance to the GPS coordinates in 2012 are the
145 descendants of the 2003 cohort. Preliminary data from >5,000 SNPs generated by genotype-by-
146 sequencing has identified a high number of independent genetic clusters in population structure
147 analyses and a low proportion of recent immigrants into populations (Alvarado-Serrano *et al*.
148 unpublished data) indicating that our assumptions herein are largely realistic. We estimated
149 population size in the 2012 sampling year by counting the numbers of individuals down a linear
150 transect.

151 Of the 26 populations that were sampled both years, we randomly chose 10 to examine
152 potential changes in genetic diversity between 2003 and 2012. One seed from an average of 18
153 maternal lines per population per sampling year (355 individuals total) were germinated and
154 cotyledons were used for DNA isolation using a CTAB method modified from Stokes et al. 2009
155 (see Kuester *et al.*2015). The numbers of maternal lines sampled per population were
156 approximately equal between the sampling years and exact numbers are presented in Table S2.

157 To assay herbicide resistance among populations and between sampling years, we planted
158 two replicate greenhouse experiments of all 26 populations at the University of Georgia Plant
159 Biology Greenhouses (Athens, GA). One seed from 10 maternal lines per population per
160 sampling year were scarified and planted in pine bark soil in SC10 super conetainers (Stuewe
161 and Sons, Tangent, OR) in six experimental treatments, described below. This design was

162 replicated in its entirety in another greenhouse for a total of 20 seeds per population within each
163 treatment and thus an overall total of 5381 experimental individuals. Plants were randomly
164 assigned to racks that were then randomly assigned to flow trays (4 racks per flow tray).
165 Conetainers were watered daily and flow trays were filled with water to prevent desiccation.
166 Germination was slightly higher in 2003 compared to 2012 samples (87% and 84% in 2003 and
167 2012, respectively, $\chi^2_1 = 12.27$, $P < 0.001$) and ranged from 50-98% across populations.

168 Plants were sprayed with RoundUp PowerMax (Monsanto, St Louis, MO) 22 days after
169 planting at rates around the recommended field rate (1.54 kg ai/ha) of 0, 0.21, 0.42, 0.84, 1.70
170 and 3.40 kg a.i./ha (the 0 kg a.i./ha control treatment was sprayed with water) using a hand-held,
171 CO_2 pressurized sprayer (R & D Sprayers, Opelousas, LA) that delivered 187 L ha^{-1} at 206 kPa,
172 1.5 meters above the plants. Three weeks after glyphosate application we scored survival of each
173 plant. Plants were harvested, dried at 72°C for 48 hours and measured for total above ground
174 biomass. Biomass values were adjusted to the non-sprayed controls by dividing each individual
175 by the average biomass of its population grown in the non-spray control treatment following
176 standard protocols (Tehranchian *et al.* 2015).

177 **SSR genotyping and scoring errors.** Details on multiplexing SSR markers and scoring
178 procedures can be found in Kuester *et al*. (2015). Briefly, 15 polymorphic microsatellite loci
179 were used to examine genetic diversity across populations and sampling years, and all
180 individuals were scored by hand. To check accuracy of multi-locus genotypes we re-scored loci
181 from 200 randomly chosen individuals and found very few scoring errors. We did not find any
182 large allele drop-outs or errors due to stutter in any of the locus by population by year
183 combinations. We also examined the influence of null alleles on genetic diversity and found little

evidence that potential null alleles altered our estimates or the main conclusions. Details of these analyses are presented in the Supporting Information section.

Temporal genetic differentiation and diversity. We examined the potential that seeds sampled across collection years were genetically differentiated from one another in two ways. First, we estimated genetic differentiation between years (F_{RT}) using hierarchical AMOVA in GenAlEx v. 6.5 (Peakall & Smouse 2012). We also performed individual assignment (Paetkau *et al.* 1995; Cornuet *et al.* 1999) of individuals to sampling year using GeneClass2 (Piry *et al.* 2004). For individual assignment, the inability to assign individuals to a specific sampling year would indicate that individuals sampled in 2012 had not diverged in allelic composition compared to the individuals sampled in 2003. We used the Bayesian method described by Baudouin and Lebrun (Baudouin & Lebrun 2000) as a criterion for computation, and individual assignment was performed using the leave-one-out procedure (Paetkau *et al.* 2004), where the genotype to be assigned was not included in the population from which it was sampled. We report the –log likelihood of being assigned in each sampled year, by plotting the –log likelihood value of individual assignment to 2003 sample year against the –log likelihood of being assigned to the 2012 sampling year. Lack of temporal change across sampling years would be indicated by overlap of individuals sampled from each year. We calculated expected and observed heterozygosity (H_e and H_o), the number of alleles (Na) and the number of effective alleles (Ne) using GenalEx v 6.5 (Peakall & Smouse 2012) and and allelic richness (AR) using FSTAT v. 2.9.3.2 (Goudet 2005) and determined if there were reductions in diversity estimates between 2003 and 2012 using Wilcoxon matched pairs rank sum tests (Zar 1996). We estimated the inbreeding coefficient (F_{IS}) of each population in each sampling year using GenePop v 4.5.1

206 (Rousset 2008) to determine if there was evidence of inbreeding among populations and if this
207 significantly differed according to sampling year. Finally, we examined the possibility that
208 populations experienced genetic bottleneck using the program BOTTLENECK (Piry *et al.* 1999).
209 This program examines the potential for greater expected heterozygosity based on allelic
210 diversity relative to expected heterozygosity estimated under mutation-drift equilibrium (Nei *et*
211 *al.* 1975; Cornuet & Luikart 1996). If a significantly high proportion of loci exhibit an allele
212 deficiency relative to expectations based on mutation-drift equilibrium, the population would
213 show signs of a recent reduction in the effective population size and thus a bottleneck (Nei *et al.*
214 1975; Cornuet & Luikart 1996). We conditioned analyses on the infinite alleles model (IAM),
215 the step-wise mutation model (SMM) and the two-phase model (TPM) of microsatellite mutation
216 since we are using microsatellites with a range of repeat motif types—dimeric, trimeric, and
217 imperfect motifs—and thus we have no a priori reason to select one particular mutational model
218 over another (repeat types presented in STable2 of (Kuester *et al.* 2015)). All analyses were
219 performed across 1000 iterations assuming mutation-drift equilibrium, and significance was
220 calculated using the Wilcoxon test (appropriate for sample sizes of < 30 individuals, (Luikart &
221 Cornuet 1998; Luikart *et al.* 1998)).

222

223 **Resistance screen.** We examined the potential that populations and sampling years varied for
224 resistance using univariate mixed-model analyses of variance. We assessed resistance in two
225 ways—first, as a measure of the number of individuals within populations that died as a result of
226 herbicide application, and second, as a measure of the amount of biomass change following
227 herbicide application. We used the glmer option of the lme4 package in R (Bates *et al.* 2011) and

228 modeled survival as a binary character (0/1) and used the lmer option to assess biomass
229 remaining post-herbicide. In each model, replicate greenhouse experiment, herbicide treatment,
230 collection year, and population were the independent variables with survival or standardized
231 biomass as the dependent variables. We included interactions between population and collection
232 year as well as population, collection year and treatment. Population and its interaction terms
233 were considered random effects in each model whereas all other effects were fixed. We
234 previously identified a significant population effect from the 2012 cohort for survival post-
235 herbicide application, which indicated that populations vary in their respective level of resistance
236 (Kuester *et al.* 2015). Here we are specifically interested in the year term as well as interaction
237 terms including the year effect, which would indicate that resistance varies between sampling
238 years and/or that populations vary in their level of resistance between years. An F-test was used
239 to determine the significance of fixed effects, and the significance of each random effect in the
240 model was determined using a likelihood ratio test (LRT) in which the full model was compared
241 to a reduced model with the effect of interest removed. The *P*-value was determined using a $|^2$
242 test with one degree of freedom. We examined the normality of our estimates of biomass with
243 the Shapiro-Wilk test and by visual inspection of quantile-quantile (q-q) plot, and square root
244 transformed this variable to improve normality of the residuals.

245

246 **Results**

247 **Genetic diversity and differentiation**. We uncovered reductions in genetic diversity between
248 sampling years among populations (Table 1), with most measures of diversity significantly
249 reduced in 2012 compared to 2003 (Figure 2). For example, expected heterozygosity was 32%

250 lower in 2012 (W = 51, P = 0.01), allelic richness was 18% lower (W = 52, P = 0.01), the
251 effective number of alleles was 43% lower (W = 51, P = 0.01) and the absolute number of alleles
252 per locus were reduced by 19% in 2012 compared to 2003 (W = 50, P = 0.01). The observed
253 heterozygosity was 27% higher, on average, in 2012 compared to 2003 (W = 4, P = 0.005). This
254 difference is likely due to the low observed compared to expected heterozygosity of the 2003
255 cohort, *i.e.,* the inbreeding coefficient ($F_{IS} = 1 - H_o/H_e$) was higher in 2003 versus 2012 (F_{2003} =
256 0.57 ± 0.05 (±SE) vs. F_{2012} = 0.13 ± 0.04, respectively; Figure 2). The difference in average F_{IS}
257 value between 2003 and 2012 was significant (W = 55, P < 0.01). Although this difference could
258 be due to selection against heterozygotes in 2003, it is more likely indicative of differences in the
259 mating system between sampling years of this mixed-mating, hermaphroditic species.
260 Populations were sampled during a slightly longer window of time in 2003 than in 2012 (10/10-
261 11/3 in 2003 vs 10/15-10/20 in 2012); however, at least five of the 10 populations were sampled
262 during the same temporal window (10/10-10/20 both years), and these populations exhibit
263 similar differences in F values (F_{2003} = 0.47±0.08 vs. F_{2012} = 0.12±0.03). We do not have
264 information regarding pollinator abundance or any other reason to expect differences in the
265 mating system between years.

266 Bonferroni-corrected HWE tests, consequently, indicated that more loci were not in
267 HWE equilibrium within populations in 2003 (39 out of 150 locus × population combinations),
268 compared to 2012 (1 out of 150 locus × population combinations). Processes that lead to
269 heterozygote deficit, such as inbreeding or population substructure can cause deviations from
270 HWE; alternatively, the presence of null alleles could inflate estimates of homozygosity and lead
271 to deviations from HWE (Kelly *et al.* 2011). We tested for the potential that null alleles altered

272 our estimates of genetic diversity by removing loci with >25% putative null allele frequencies
273 across populations and re-estimated indices of genetic diversity. We found no evidence that loci
274 with potential null alleles altered our estimates of genetic diversity or the conclusion that
275 diversity is altered between sampling years (Table S3, Supporting Information).

276 We further examined changes in the patterns of allelic diversity by investigating the
277 number of alleles, the number of rare alleles (<10% frequency) and the frequency of rare alleles
278 present in 2003 and 2012. At the species level (*i.e.,* across all populations), we found no
279 evidence for a reduction in the total number of alleles from 2003 to 2012 (42 versus 44 alleles in
280 each year, respectively)—unexpectedly, we found fewer rare alleles in 2003 than 2012 (10 vs
281 17). Only four of the rare alleles present in 2003 were likewise present in 2012, and their
282 frequency was not dramatically increased as would be expected if rare allele frequency changes
283 were responsible for the higher observed heterozygosity in 2012. When examining the number of
284 alleles per population, however, we found that the total number of alleles was reduced in eight of
285 10 populations, by as much as 12-40% across populations. Two of the ten populations
286 (populations 26 and 28) exhibited gains of low frequency alleles (between 4-5 new alleles
287 present in 2012 at frequencies of <10%). Thus, 8 of the 10 populations show reductions in
288 diversity over time likely due to random genetic drift, whereas two of the populations exhibit an
289 increase in the number of alleles, putatively due to migration, drift, or mutation.

290 We tested for a signature of bottleneck events in both the 2003 and 2012 samples using
291 the BOTTLENECK program, and found that significance of these tests depended on both the
292 specific model employed (IAM, SMM, or TPM) and the sampling year. Under the IAM, six

293 populations sampled from 2003 exhibited significant heterozygote excess following corrections
294 for multiple tests, whereas only one – population 32 – exhibited significant heterozygote excess
295 under all three models of microsatellite evolution (Table 2). No populations from 2012 exhibited
296 evidence of heterozygote excess and thus signs of a bottleneck following corrections for multiple
297 tests (Table 2).

298 We next estimated the effective number of individuals from each sampling year using
299 expected heterozygosity and the equation $H_e = 4N_e\mu$ (Nagylaki 1998) with a mutation rate, μ, of
300 10^{-3} (Marriage *et al.* 2009). We found that the estimated number of individuals from the 2003
301 populations was significantly higher, on average, compared to that of the 2012 populations ($N_{e,}$
302 $_{2003} = 85$, $N_{e,\,2012} = 58$; W = 67, P = 0.005). Furthermore, we found no significant difference
303 between our census sample size from the 2012 populations and the estimated effective number of
304 individuals from that sampling year (Population size average from census = 70 individuals; W =
305 36.5, P = 0.32). While the difference in estimated number of individuals between sampling years
306 indicated that most populations experienced reductions in size (reductions ranging from 20-55
307 individuals fewer in 2012), populations 26 and 28 both exhibited an estimated gain of 20
308 individuals.

309 In line with lower diversity of the majority of populations, we found significant genetic
310 differentiation between individuals sampled from different collection years (AMOVA year
311 effect, $F_{RT} = 0.218$, P = 0.001, Table S4), and evidence that individuals sampled as seed in 2003
312 were more similar to one another than to individuals sampled as seed from the same location in
313 2012 (Figure 3), *i.e.,* no individual assigned to 2003 was likewise assigned to 2012. We found

314 that the estimate of F_{RT} was inflated by loci that potentially harbored null alleles; however, after
315 removing these loci from analysis, we found that the F_{RT} estimate was still significantly different
316 from zero, indicating the presence of between-year genetic differentiation ($F_{RT(8\ loci)} = 0.133$, P =
317 0.001, Table S4). We did not remove loci with potential null alleles from genotypic assignment
318 as these tests are not greatly influenced by their presence (Carlsson 2008).

319 **Phenotypic divergence.** We examined resistance traits (survival and biomass post-herbicide
320 application) to determine if there was evidence of changes in resistance between sampling years.
321 Our mixed-effects analyses of variance uncovered a significant year effect for biomass remaining
322 after herbicide application ($F_{1, 3595} = 4.72$, P = 0.03; Table 3). On average across all populations,
323 the biomass remaining post-spray of the 2012 cohort was slightly greater than that of the 2003
324 cohort (62% vs. 57% in 2012 and 2003, respectively) suggesting moderate increases in resistance
325 across populations sampled in 2012 (see Table S5 for averages (±SE) among all populations).
326 Likewise, a higher percentage of individuals sampled in 2012 survived herbicide application
327 compared to those sampled from 2003 (49% vs 42%), but this difference was not significant ($F_{1,}$
328 $_{5365} = 2.58$, P = 0.11; Table 3).

329 As in previous work (Kuester *et al.* 2015), we identified significant population effects for
330 both measures of resistance (Table 3), indicating that populations vary across the landscape for
331 their relative level of herbicide resistance. Here, however, we also find population by year effects
332 in each analysis, indicating that populations differ in their level of resistance across years
333 (Survival, $\chi^2 = 23.74$, P < 0.001; Biomass, $\chi^2 = 7.92$, P = 0.005; Table 3). One population's
334 survival increased by 79% compared to 2003, indicating that some populations may respond
335 more readily with increased resistance than others. In general, three populations sampled from

336 TN that were highly resistant in 2012 (Kuester *et al.* 2015) were similarly resistant in 2003
337 (Figure 1 A & B, shown at 2X field rate of RoundUp). The majority of the significant increases
338 identified in the 2012 cohort compared to the 2003 cohort were located in NC and SC (Figure 1
339 A & B)—while five populations from the 2012 cohort of this region exhibit resistance values
340 significantly greater than the species-wide average (56% survival at 2X the field rate of
341 RoundUp (Kuester *et al.* 2015)), the 2003 cohorts of these populations exhibited only ~14%
342 survival at 2X field rate.

343

344 **Discussion**

345 Despite the ubiquity and persistence of weedy plant populations, there are few
346 examinations of how their neutral and adaptive genetic diversity may change over time. Here we
347 use a resurrection ecology experiment to show that populations of weedy *I. purpurea* sampled
348 from crop fields concomitantly lose genetic diversity and show signs of potential adaptive
349 evolution in herbicide resistance. Our experiments yielded three novel findings. First, we found
350 that seed progenies from populations sampled in 2012 exhibited lower genetic diversity and
351 higher genetic differentiation than seed progenies sampled from the same fields and locations in
352 2003, suggesting that populations have experienced a genetic bottleneck between sampling
353 periods. Second, heterozygosity excess tests indicated that a significant genetic bottleneck likely
354 also occurred prior to 2003, perhaps due to the dramatic increase in glyphosate use in the late
355 1990's (see Fig 1 in Baucom & Mauricio 2004). Although we cannot ascribe the loss of neutral
356 genetic variation to the widespread use of herbicide *per se*, we show that a resistance trait—the
357 amount of biomass maintained following herbicide application—has increased in some

358 populations from 2003 to 2012. We combine these results with a retrospective analysis of a
359 larger and previously published dataset showing that highly resistant populations sampled in
360 2012 exhibit significantly reduced heterozygosity and allelic richness estimates compared to less
361 resistant populations. Below we discuss each of these major findings.

362 *Reductions in genetic diversity between sampling years*

363 We currently have a very limited understanding of how agricultural regimes may
364 influence the population genetics of agricultural weeds. Although processes such as tilling and
365 herbicide use are hypothesized to result in genetic bottlenecks, previous examinations of
366 agricultural weed populations have either failed to uncover substantial reductions in genome-
367 wide diversity (Kane & Rieseberg 2008) or have presented largely circumstantial evidence for
368 bottlenecks (i.e., comparisons between species; (Hamrick *et al.* 1979)). The significant loss in
369 diversity that we uncovered across populations of *I. purpurea* sampled from agricultural fields
370 argues for a bottleneck that was either very strong or occurred more than once, or both. While
371 there are no studies, to our knowledge, that examine the temporal genetics of agricultural weed
372 populations for comparison, it is of note that the average loss of allelic richness that we identified
373 across populations (on average 15% lower between cohorts) is similar in magnitude to that of
374 introduced, colonizing species (18% loss compared to native populations (Dlugosch & Parker
375 2008)). Furthermore, using expected heterozygosity estimates from each sampling year, we find
376 that the estimated population sizes have reduced between 2003 and 2012, with the majority of
377 the populations losing reproductive individuals. While we did not take population census data in
378 2003 for comparison, we find that the estimated population size in 2012 is not significantly
379 different from the census size, suggesting that our estimated population sizes are decent

380 approximations of the true census size. The majority of the populations exhibited loss of alleles
381 between sampling years, however, two populations—#26 and #28—exhibited gains of low
382 frequency alleles, and the estimated sample size of these two populations likewise increased
383 relative to other populations. The increased diversity of these populations likely were from
384 dormant seeds, or, alternatively, due to migration from another population. Emergence of seed
385 stored in the seed bank is incredibly likely—this species can produce a large number of seeds in
386 field conditions (between 3,000-10,000 per individual), and these heavy, gravity dispersed seeds
387 can remain dormant for ~20 years in the soil (Baskin & Baskin 2000).

388 Interestingly, while the loss of allelic diversity between 2003 and 2012 suggests a genetic
389 bottleneck has occurred between sampling years, the within-population examination of
390 heterozygosity excess (*i.e*, the bottleneck test) did not find evidence of genetic bottleneck in the
391 2012 populations. Instead, our tests of heterozygosity excess uncovered evidence of genetic
392 bottleneck among six populations collected in 2003 under the IAM model, with one population
393 exhibiting evidence of a genetic bottleneck under all three models of microsatellite evolution.
394 Other studies using heterozygosity excess tests have reported limited support of genetic
395 bottleneck in species known to have experienced population declines (Hufbauer *et al.* 2004;
396 Peery *et al.* 2012) with the general conclusion that heterozygosity-excess tests may be limited to
397 severely bottlenecked populations (Peery *et al.* 2012). Our results suggest that a genetic
398 bottleneck occurred in some populations prior to the 2003 sampling, possibly following the sharp
399 increase in the widespread use of glyphosate across RoundUp Ready crops (see Fig 1 in Baucom
400 & Mauricio 2004). In this scenario, the loss of allelic diversity that we identified in 2012 may
401 simply be a continuation of effects following the initial population bottleneck. Alternatively, it is

402 possible that demographic bottlenecks occurred prior to 2003 and continued between 2003 and
403 2012, but to a lesser extent between years. While a few of the 2012 populations did exhibit
404 heterozygosity excess, defined as greater heterozygosity based on estimates of allelic diversity
405 relative to heterozygosity estimated under mutation-drift equilibrium, tests were not significant
406 following corrections.

407 Another attribute of the data suggest populations experienced a genetic bottleneck prior
408 to 2003—we found more locus by population combinations out of HWE compared to 2012. In
409 addition to the evolutionary forces of genetic drift, selection, mutation, migration, and non-
410 random mating (i.e. inbreeding or non-assortative mating), null alleles can also cause deviations
411 from Hardy-Weinberg equilibrium and will appear as heterozygote deficiency (Dąbrowski *et al.*
412 2014). The pattern that we uncovered of low observed heterozygosity relative to expected
413 heterozygosity in our 2003 populations is consistent with the presence of null alleles but also
414 consistent with high levels of inbreeding. To investigate the potential that null alleles influenced
415 our results, we removed loci with putative null alleles from analyses and found that estimates of
416 genetic diversity remained lower in the 2012 populations compared to their 2003 counterparts;
417 further, F indices remained significantly higher in the 2003 sample, implicating wide-spread
418 inbreeding following a potential bottleneck. *I. purpurea* is a hermaphroditic species that displays
419 a wide range of outcrossing rates in nature (t_m range: 0.2-0.8, across 20 populations; Kuester et
420 al. *unpublished data*), thus it is plausible that inbreeding could follow a large demographic
421 bottleneck in this species. The majority of our loci exhibited $F_{IS} > 0$ in 2003 across populations,
422 further pointing to a scenario of inbreeding following a large demographic change rather than the
423 influence of null alleles. Finally, it is of note that null allele detection methods have been shown

424 to exhibit low reliability when applied to non-equilibrium populations and will overestimate their
425 frequency when populations have recently experienced demographic bottleneck (Dąbrowski *et*
426 *al.* 2014).

427 *Pattern of phenotypic variation between temporal samples*

428 Recent work provides an interesting contrast between the phenotypic and neutral genetic
429 variation spatially distributed within this system—while neutral genetic differentiation among 44
430 *I. purpurea* populations is low (*i.e.,* $F_{ST} = 0.127$), populations are significantly differentiated for
431 herbicide resistance across the landscape, with some populations exhibiting high and others very
432 low resistance (Kuester *et al.* 2015). Our screen of herbicide resistance in 26 of these temporally
433 sampled populations shows that, in addition to a mosaic of resistance across the landscape, the
434 level of resistance has slightly increased, on average, between sampling dates. This finding is
435 interesting in light of the reduced neutral genetic variation that we identified in eight of our 10
436 temporally sampled populations, and alternatively, in light of evidence of potential migrants in
437 two of the 10 populations—reductions in diversity as well as influx of presumably non-adapted
438 variation would either act to impede or to counteract adaptation. These forces, along with recent
439 work showing a severe fitness penalty of herbicide resistance in this species (Van Etten *et al.*
440 2015) likely explain why the average increase in resistance that we identified among all
441 populations was modest—perhaps the populations that maintained resistance between sampling
442 years (TN populations) or those that exhibit large increases in resistance (NC/SC populations)
443 were less influenced by susceptible migrants germinating from the seed bank and/or costs of
444 resistance than other populations. The low average increase in resistance across populations
445 could also be due to a range of other factors: it is possible that few populations house additive

446 genetic diversity for resistance, populations may experience different selective regimes, or the
447 response to selection via glyphosate has been constrained by bottleneck events. Interestingly,
448 there was no evidence that plants were different in size between the years (*data not shown*),
449 indicating that the increased resistance we detected is not due to plants simply being larger in the
450 2012 cohort and thus better able to withstand herbicide application.

451 Although we find evidence for a moderate increase in the level of resistance across
452 populations, it is important to note that our phenotypic comparisons were made using field-
453 collected seeds. Thus our resistance estimates include both potential genetic components and
454 environmental effects. This could explain the slight increase in resistance over time: if more
455 2012 populations had experienced glyphosate application relative to the 2003 populations, we
456 would perhaps be sampling from a subset of the population that experienced herbicide relative to
457 plants that had not, potentially inflating estimates of resistance in the 2012 samples. In 2003,
458 between 80-92% of the soy fields in the US were RoundUp Ready, and thus sprayed with the
459 herbicide, whereas approximately 20% of corn was RoundUp Ready in that sampling year. In
460 2012, 93% of soy planted was RoundUp Ready and between 73-80% of corn was RoundUp
461 Ready (NASS 2015). Thus, it is possible that our comparison of the temporally sampled
462 phenotypes is influenced by exposure to the herbicide itself in 2012. We considered this by using
463 crop type as a proxy for herbicide use, and determined if biomass remaining post-spray differed
464 according to crop (restricted to soy and corn) separately for both 2003 and 2012. There was no
465 difference in biomass remaining post-herbicide according to crop either year—e.g., the biomass
466 remaining post-herbicide of morning glories sampled from soy was no different than those
467 sampled from corn—suggesting that the population-level estimates of resistance are not

468 dependent on the crop type (as a proxy for spraying regime) in one particular sampling year.
469 While we cannot conclude that the increase in our field estimates of resistance between sampling
470 years is solely due to adaptive evolution, we have previously identified an additive genetic basis
471 underlying glyphosate resistance in one population of this species (Baucom & Mauricio 2008),
472 positive selection for increased resistance in the field (Baucom & Mauricio 2008), and further,
473 have shown that resistance segregates in crosses (Debban *et al.* 2015) indicating that the genetic
474 potential is present within at least one population. Identification of the genetic basis of resistance
475 across populations, and an assessment of how alleles associated with resistance change over time
476 will decisively test our hypothesis that selection from the use of this herbicide is leading to
477 adaptation in natural populations.

478 *Has herbicide application caused the genetic bottleneck?*

479 The populations used in this study were all sampled from crop fields that were farmed
480 prior to and from 2003 onward. While we do not have specific information on herbicide use over
481 this time period, we have historical record for six of 10 years (Table S1) showing that these
482 locations were used for corn and soy crops, both of which make use of herbicides—and largely
483 glyphosate—for weed control. Although other environmental factors (e.g., those associated with
484 climate change) could be responsible for the genetic bottleneck that we report herein, herbicide
485 use is an obvious potential factor. We examined this idea by making use of a larger and
486 previously published dataset of 32 populations, sampled in 2012, for which we have estimates of
487 both resistance and genetic diversity (Kuester *et al.* 2015). We performed separate regressions of
488 expected heterozygosity (He) and allelic richness (AR) on estimates of herbicide resistance
489 (proportion survival) at 3.4 kg ai/ha of glyphosate and found a significant negative relationship,

490 *i.e.,* more resistant populations exhibit lower genetic diversity (He vs resistance: R = -0.375, P =
491 0.04; AR versus resistance: R = -0.345, P = 0.05). There is thus some indication that selection
492 via herbicide application has led to the genetic bottleneck among populations. Interestingly, a
493 population used in both analyses – population 32 – exhibits high survival at 3.4 kg ai/ha of
494 glyphosate, and was the population for which we found evidence of a pre-2003 bottleneck under
495 all models of microsatellite evolution.

496

497 Conclusions—Weedy plant species found in agricultural fields experience strong selection and
498 thus are hypothesized to be either plastic, capable of adaptation, or saved from extinction through
499 gene flow (Baker 1974; Parker *et al.* 2003). By using a resurrection ecology framework, we
500 provide evidence that even though genetic variation is lost from the system, some populations
501 show potential signs of adaptation to herbicide application. While previous work indicates that
502 the majority of the gene flow across southeastern populations occurred prior to the wide-spread
503 adoption and use of glyphosate, suggesting that resistance evolution is due to selection on
504 standing or novel variation within populations, that we identified evidence of potential migrants
505 into the 2012 gene pool (in at least two populations) does not allow us to rule out the hypothesis
506 that resistance can be introduced from outside sources.

507 Further, while we find evidence of increased resistance, we also show that the absolute
508 change between years was not drastic; large resistance gains were limited to particular
509 populations. These data suggest heightened measures should be taken to reduce the likelihood
510 that seeds are accidentally moved between crop fields through farm machinery or through
511 contaminated seed lots. Finally, we have some evidence that the lower genome-wide diversity

identified across populations is due to the application of glyphosate; however, we note that we cannot rule out other potential factors, since other herbicides with different mechanisms of action are often applied in crops, other cropping techniques that reduce population sizes might have been employed, and it is also possible that populations have lost diversity due to changes in the climate. The results shown here suggest that this weed, while being a 'general purpose genotype' (Baker 1974; Chaney & Baucom 2014) is also capable of adaptive evolution even while losing significant allelic diversity. How likely future adaptation to novel selective forces may be in the future, in light of reduced variation, is unknown.

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661 **Data Accessibility**.

662 SSR Genotyping and morphological data is available on Dryad, doi to be provided upon
663 acceptance.

664 Sampling locations and crop history information is provided in the Supplemental materials
665 section.

666

667

668

Tables

Table 1. The genetic diversity of populations between sampling years. Shown are the number of alleles (Na), the effective number of alleles (Ne), the observed and expected heterozygosity (Ho and He, respectively), allelic richness (AR), and the inbreeding coefficient (F_{IS}) of each population.

	Na		Ne		Ho		He		AR		F_{IS}	
Population	2003	2012	2003	2012	2003	2012	2003	2012	2003	2012	2003	2012
2	2.27	1.93	1.53	1.37	0.09	0.17	0.30	0.22	1.96	1.68	0.71	0.24
8	2.33	1.67	1.76	1.31	0.17	0.18	0.38	0.17	2.16	1.48	0.59	-0.06
10	2.20	1.93	1.65	1.29	0.13	0.15	0.33	0.18	1.97	1.59	0.63	0.18
18	2.40	1.47	1.76	1.27	0.13	0.15	0.36	0.16	2.05	1.41	0.66	0.08
21	2.53	1.40	1.90	1.14	0.14	0.12	0.41	0.18	2.29	1.53	0.69	0.39
23	2.53	1.93	1.84	1.46	0.16	0.24	0.39	0.27	2.18	1.75	0.59	0.15
26	1.80	2.13	1.39	1.61	0.18	0.24	0.23	0.31	1.61	1.87	0.25	0.21
28	2.13	2.40	1.40	1.55	0.15	0.28	0.23	0.31	1.71	1.94	0.36	0.09
30	2.27	1.93	1.86	1.48	0.19	0.27	0.40	0.28	2.14	1.73	0.64	0.10
32	2.40	1.80	1.73	1.43	0.14	0.23	0.37	0.24	2.14	1.66	0.55	0.03

Table 2. Tests of heterozygosity excess within populations for each sampling year using the BOTTLENECK program (Cornuet & Luikart 1996). Tests were performed using three different models of microsatellite evolution, each of which assumes mutation-drift equilibrium (IAM, infinite alleles model; SMM, stepwise mutation model; TPM, two-phase model. Probability values from one-tailed Wilcoxon tests are shown, with bolded values indicating statistical significance following corrections for multiple tests (P < 0.005).

	2003			2012		
Population	IAM	SMM	TPM	IAM	SMM	TPM
2	0.117	0.810	0.396	0.313	0.615	0.539
8	**0.002**	0.084	0.020	0.230	0.527	0.422
10	0.016	0.249	0.047	0.765	0.945	0.867
18	**0.004**	0.188	0.047	0.027	0.055	0.055
21	**0.003**	0.122	0.016	0.371	0.473	0.371
23	**0.003**	0.084	0.016	0.095	0.271	0.249
26	0.117	0.396	0.235	0.122	0.249	0.153
28	0.485	0.867	0.715	0.249	0.773	0.580
30	**0.003**	0.227	0.055	0.012	0.216	0.138
32	**<0.001**	**0.004**	**0.001**	0.032	0.170	0.133

Table 3. Generalized linear mixed effects model of resistance in *I. purpurea*. Models include fixed effects of experimental replicate, treatment, sampling year, sampling x treatment interaction; population and interactions of population x year, population x treatment, and population x treatment x year are considered random effects.

	Survival			Biomass		
Effect	Df	F	P	Df	F	P
Fixed Effects						
Replicate	1	14.46	<0.001	1	12.58	<0.001
Treatment	5	154.74	<0.001	5	190.98	<0.001
Year	1	2.58	0.108	1	4.72	0.030
Year x Treatment	5	0.09	0.994	5	0.86	0.511
Random Effects		X^2			X^2	
Population	1	19.18	<0.001	1	4.97	0.026
Population x Year	1	23.75	<0.001	1	7.92	0.005
Population x Treatment	1	3.70	0.054	1	<0.001	1
Population x Treatment x Year	1	<0.001	1	1	<0.001	1
Residual DF	5365			3595		

Figures

Figure 1. Map of populations sampled from A) 2003 and B) 2012 within the US. Populations that were genotyped in both 2003 and 2012 are indicated by a triangle (see Table S1 for sites used for resistance measurements). The percent survival following 3.4 kg ai/ha of RoundUp is indicated in color. Sites were sampled at least 5 km apart.

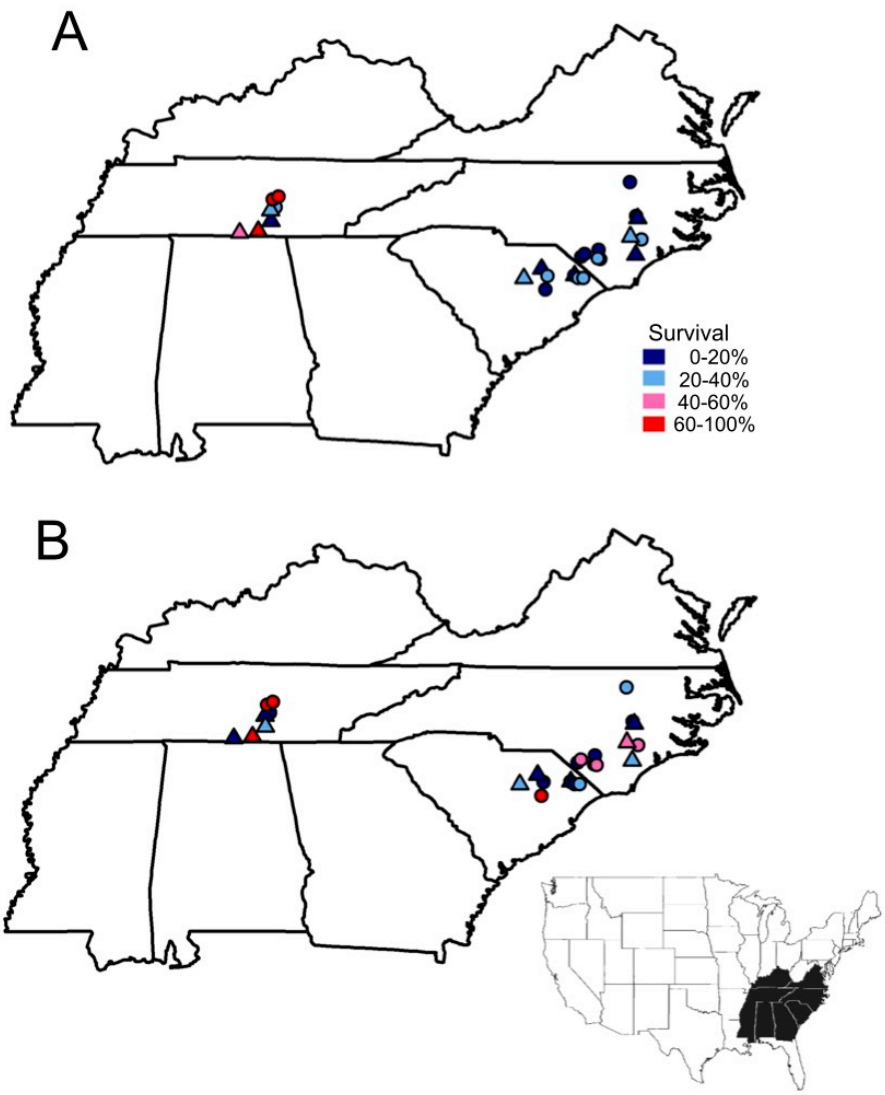


Figure 2. Genetic diversity indices compared between sampling years (2003 and 2012). Shown are the median (thick line) and lower and upper quartiles for (A) number of alleles (Na), (B) effective number of alleles (Ne), (C) observed heterozygosity (Ho), (D) expected heterozygosity (He), (E) allelic richness (AR), and (F) inbreeding coefficients (F_{IS}).

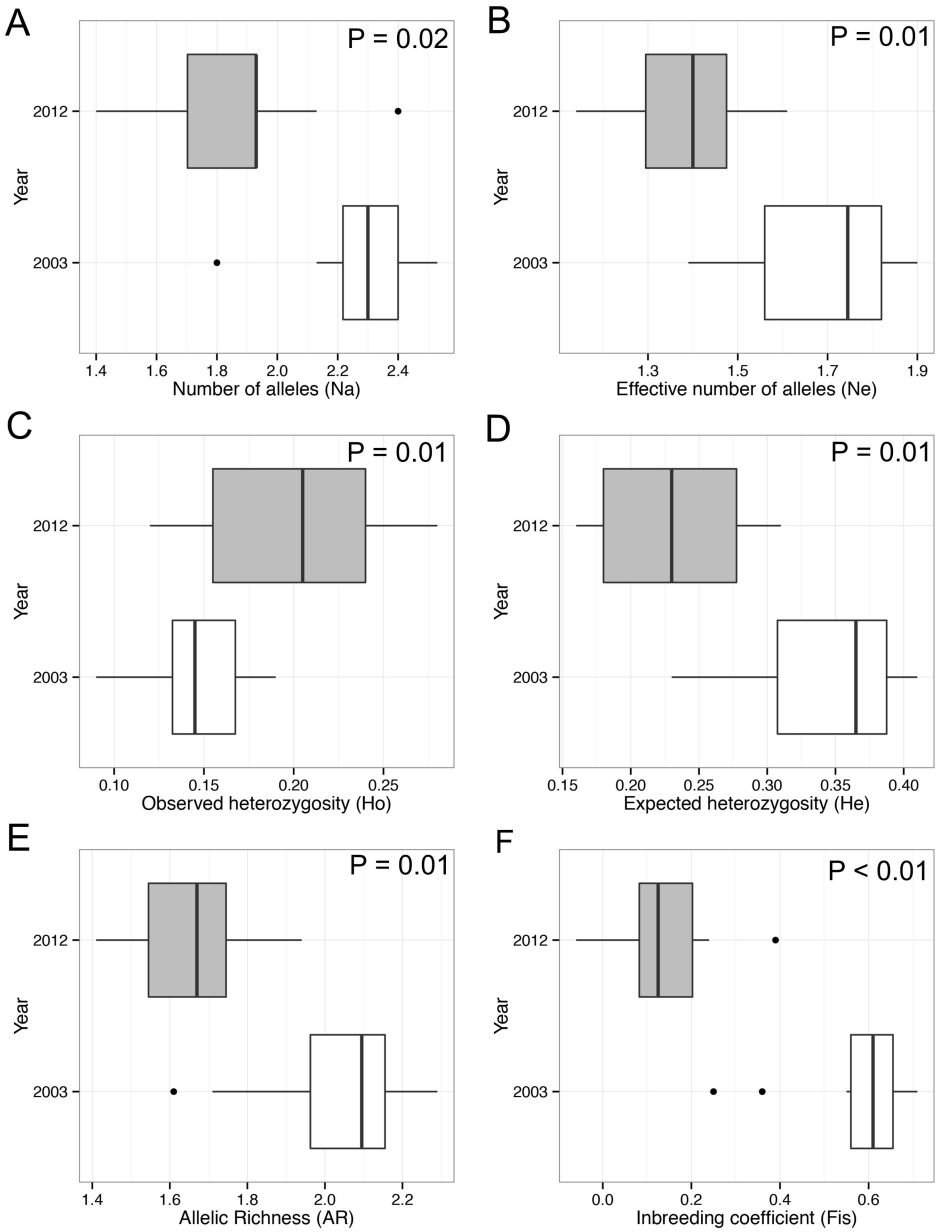
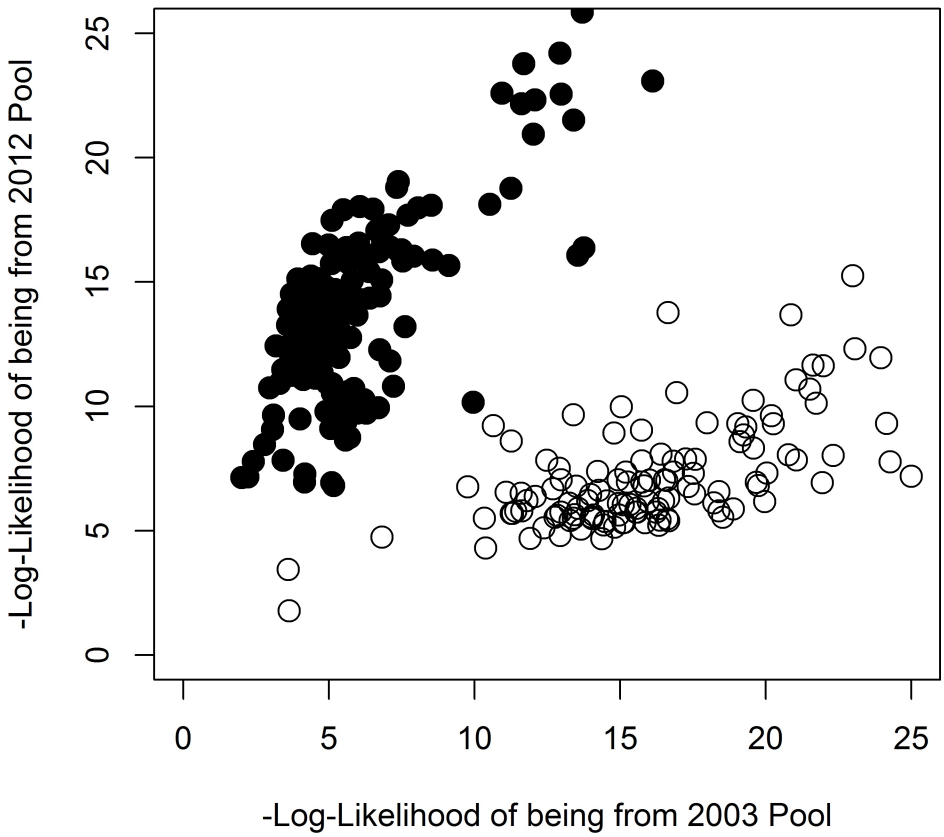


Figure 3. Scatter plots of log likelihood values from assignment tests of individual *I. purpurea* plants sampled in 2003 and 2012 based on genotypic data at 15 microsatellite loci. A higher position relative to the y-axis indicates a higher likelihood of being from 2012 pool of individuals and a higher position relative to the x-axis indicates greater likelihood of being from 2003 pool of individuals.



Supporting Information

Materials and Methods

Scoring accuracy and allelic composition of populations/sampling years—We estimated the scoring error rates per locus by randomly selecting and re-scoring the genotypes of 200 individuals. We found our scoring error rate to be very low across loci: IP31 (0%), IP2 (3.7%), IP27 (0%), IP8 (0%), IP34 (0%), IP1 (0%), IP36 (6.52%), IP47 (1.45%), IP12 (1.45%), IP21 (5.07%), IP6 (1.45%), IP45 (2.17%), IP26 (2.17%), IP42 (3.62%).

The total number of alleles observed per locus within 184 individuals sampled in 2003 and 171 individuals sampled in 2012 samples ranged from 2 to 7 in 2003 and 1 to 7 in 2012. Overall, we found a total of 42 alleles scored across 15 loci in 2003 and 44 alleles in 2012. On average there were 2.3 alleles per locus x population combination in 2003 and 1.9 alleles in 2012. The size range of alleles ranged roughly between 90 and 300 base pairs.

Hardy-Weinberg Equilibrium, scoring errors, and null alleles—Deviations from Hardy-Weinberg equilibrium (HWE) were tested using GenePop v 4.5.1 (Rousset 2008). After performing Bonferroni corrections (Rice 1989) for multiple tests, we found 39 locus x population combinations that departed from HWE in the 2003 and 1 locus x population combination that deviated from HWE in the 2012 data, respectively, out of 150 possible locus x population combinations. In 2003, the number of loci out of HWE per population ranged from 0-8 (median: 2); the departures in HWE among loci were not consistent across populations. Small population size, non-random mating, mutations, migration, and selection can cause departures in HWE; furthermore, null alleles can lead to heterozygote deficiency, which may be detected as departures from HWE. Therefore, we further explored the data to determine if null alleles and other genotyping errors were responsible for the higher number of loci out of HWE among the 2003 compared to 2012 populations.

We assessed scoring errors per locus x population combination using MicroChecker v 2.2.3 (Van Oosterhout et al. 2004) and GenePop v 4.5.1 (Rousset 2008). Large allele dropout (under-amplification of larger allelic variants) and scoring errors due to stutter (an error resulting from slippage of the polymerase during PCR) were not detected in our dataset. MicroChecker v 2.2.3 (Van Oosterhout et al. 2004) identified more loci with potential null alleles in the 2003 data compared to the 2012 data (8 of 15 versus 3 of 15 loci, respectively). We examined the frequencies of potential null alleles across loci × population combinations using GenePop v 4.5.1 and identified loci (IP2f, IP27f, IP18f, IP12f, IP21f, IP26f, IP42f) with null alleles at a frequency of >25%, on average, across populations. We removed these loci from both the 2003 and 2012 datasets (leaving eight loci: IP31, IP8, IP34f, IP1f, IP36, IP47f, IP6f, IP45f) and estimated expected heterozygosity, allelic richness, effective and absolute number of alleles using GenAlEx v. 6.5 (Pekall and Smouse 2012). All diversity estimates remained significantly lower in the 2012 data (Table S3) indicating that null alleles were not responsible for the reduced genetic variation in the 2012 populations compared to the 2003 populations. We likewise estimated genetic differentiation between years using hierarchical AMOVA in GenAlEx v. 6.5

(Pekall and Smouse 2012) and found that, as expected based on previous reports (Carlsson 2008), our F_{RT}, or measure of between-year differentiation, was potentially inflated by the seven loci with potential null alleles (Table S4, A and B: 15 loci $F_{RT} = 0.218$; 8 loci $F_{RT} = 0.133$). We elected to present results using all 15 loci in this work given that our patterns of diversity loss remained when these seven loci were removed from analysis, and in light of reports showing that bottleneck events lead to false-positives in null allele detection methodologies (Dabrowski et al. 2014). We did not remove loci with potential null alleles when performing assignment testing since null alleles do not significantly alter the outcome of such assignments (Carlsson 2008).

Linkage disequilibrium—We tested the presence of linkage disequilibrium between locus pairs for each population x sampling year combination using GenePop v 4.5.1 (Rousset 2008). All results were adjusted for multiple comparisons using Bonferroni correction (Rice 1989). Of 105 tested paired locus tests over either 2003 or 2012 sampled populations for linkage disequilibrium, 36 were found significant in 2003, though no disequilibrium was uncovered in 2012 ($P < 0.05$). The majority of locus pairs in disequilibrium from 2003 were from population 26 (25 significant pairs).

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Supplementary Tables

Table S1. Site and location information for *I. purpurea* populations used in genetic, growth and herbicide resistant assays. Shown are the population number, state of each population, the crop present in the field from 2008 to 2012, along with Latitude and Longitude GPS coordinates.

Population Number	State	Field Type						Latitude	Longitude
		2003	2008	2009	2010	2011	2012		
2	NC	soy	corn	soy	corn	soy	corn	34.595714	-77.927484
4	NC	soy	corn	soy	corn	soy	corn	34.556672	-79.125602
9	NC	soy	na	soy	soy	soy	soy	34.924044	-77.796171
10	NC	soy	corn	corn	soy	corn	soy	34.983161	-78.039309
11	NC	soy	corn	soy	corn	soy	corn	34.527135	-78.756704
14	NC	na	cotton	soy	soy	soy	soy	35.424763	-77.917121
19	NC	soy	corn	soy	corn	tobacco	corn	34.508193	-78.70899
21	NC	soy	cotton	soy	corn	cotton	soy	35.369816	-77.877314
22	NC	soy	soy	cotton	corn	corn	corn	36.1436	-78.053422
25	NC	soy	soy	soy	fallow	soy	corn	34.616361	-79.051667
29	NC	soy	corn	soy	corn	soy	corn	34.705135	-78.738897
5	SC	soy	soy	soy	soy	corn	soy	33.859875	-79.909072
8	SC	na	corn	soy	corn	soy	corn	34.297195	-79.991259
12	SC	soy	soy	corn	soy	cotton	Cotton	34.145812	-79.865313
15	SC	soy	soy	soy	peanut	soy	soy	34.104209	-79.073735
16	SC	soy	fallow	fallow	fallow	peanut	Alfalfa	34.10535	-79.183234
17	SC	soy	soy	soy	soy	soy	soy	34.159155	-79.272908
18	SC	soy	soy	soy	corn	soy	corn	34.156593	-79.27027
28	SC	soy	cotton	corn	soy	cotton	corn	34.097917	-80.377715
47	SC	na	soy	corn	soy	cotton	soy	34.282132	-79.746597
1	TN	corn	alfalfa	alfalfa	alfalfa	soy	corn	35.775237	-85.903419
20	TN	soy	soy	soy	soy	soy	corn	35.830692	-85.777871
23	TN	soy	corn	soy	corn	soy	corn	35.067905	-86.62955
26	TN	soy	corn	soy	soy	corn	soy	35.533413	-85.951902
30	TN	corn	corn	soy	corn	soy	corn	35.31105	-85.945003
31	TN	corn	soy	corn	soy	soy	corn	35.608482	-85.846379
32	TN	soy	soy	soy	soy	soy	corn	35.099356	-86.225509

Table S2. Sampling information for temporally-sampled populations of *Ipomoea purpurea*. Shown are the population identification numbers and sample sizes by year (2003 or 2012) for the SSR genotyping and resistance assays.

	SSR Sample Size		Resistance Assay Sample Size	
Population Number	2003	2012	2003	2012
2	16	15	113	101
4	--	--	117	115
9	--	--	113	87
10	17	26	115	68
11	--	--	90	116
14	--	--	115	116
19	--	--	113	106
21	16	6	116	109
22	--	--	114	107
25	--	--	116	100
29	--	--	113	112
5	--	--	118	69
8	13	20	110	107
12	--	--	116	117
15	--	--	105	118
16	--	--	105	112
17	--	--	114	118
18	22	15	115	115
28	27	18	109	117
1	--	--	63	62
20	--	--	59	61
23	24	17	80	110
26	18	16	108	115
30	12	20	115	115
31	--	--	110	105
32	19	18	66	75

Table S3. The genetic diversity of populations between sampling years with loci with putative null alleles removed (retaining 8 of the original 15). Shown are the number of alleles (Na), the effective number of alleles (Ne), the observed and expected heterozygosity (Ho and He, respectively), and the fixation indices estimated by GeneAlEx v 6.5 (Pekall and Smouse 2012).

	Na		Ne		Ho		He		F	
Population	2003	2012	2003	2012	2003	2012	2003	2012	2003	2012
2	2.286	2.143	1.634	1.386	0.161	0.267	0.338	0.224	0.478	-0.145
8	2.571	1.714	1.892	1.424	0.221	0.328	0.421	0.211	0.454	-0.419
10	2.143	2.000	1.529	1.313	0.218	0.225	0.295	0.198	0.429	-0.108
18	2.714	1.571	1.840	1.439	0.214	0.267	0.364	0.246	0.463	-0.041
21	2.857	1.714	2.112	1.305	0.179	0.262	0.451	0.212	0.523	-0.227
23	2.857	2.000	1.970	1.551	0.269	0.353	0.432	0.308	0.367	-0.056
26	2.000	2.143	1.530	1.488	0.246	0.366	0.316	0.269	0.207	-0.203
28	2.286	2.714	1.548	1.504	0.231	0.413	0.319	0.303	0.157	-0.131
30	2.571	1.857	1.774	1.492	0.173	0.379	0.374	0.272	0.451	-0.167
32	2.429	2.143	1.864	1.467	0.250	0.379	0.376	0.274	0.425	-0.143
Average	2.471	2.000	1.769	1.437	0.216	0.324	0.369	0.252	0.395	-0.164
Wilcoxon paired rank sums	**<0.01**		**<0.01**		**<0.01**		**<0.01**		**<0.01**	

Table S4. Analysis of Molecular Variance (AMOVA) of neutral genetic data estimated using GeneAlEx v 6.5 (Pekall and Smouse 2012). Shown separately for A) 15 and B) 8 loci are the main effects of sampling year (2003 vs 2012), population nested within year and individuals nested within populations, and F and P values for each effect.

A.

Source	df	F-Statistics	Value	P
Year	1	Frt	0.218	0.001
Population(Year)	18	Fsr	0.179	0.001
Individual (Population)	335	Fst	0.358	0.001
Individual (Total)	355	Fis	0.522	0.001
Total	709	Fit	0.693	0.001

B.

Source	df	F-Statistics	Value	P
Year	1	Frt	0.133	0.001
Population(Year)	18	Fsr	0.167	0.001
Individual (Population)	335	Fst	0.278	0.001
Individual (Total)	355	Fis	0.320	0.001
Total	709	Fit	0.509	0.001

Table S5. Mean and standard error for (A) resistance traits (survival and above-ground biomass at 1.7 kg a.i./ha glyphosate) and (B) size traits (plant height and number of leaves) for each population x year combination. Survival was calculated as the number of individuals per population that survived glyphosate application divided by the total number of individuals within the population. Populations 1, 20 and 32 were removed from analysis of biomass remaining post-herbicide due to very low sample size (range: 0-6 per treatment).

	Survival		Biomass			
	2003	2012	2003		2012	
PopID	Proportion	Proportion	Mean	SE	Mean	SE
1	1.000	1.000	--		--	
2	0.278	0.353	0.575	0.120	0.395	0.051
4	0.158	0.211	0.430	0.035	0.466	0.058
5	0.263	0.727	0.479	0.051	0.449	0.075
8	0.647	0.529	0.755	0.130	0.688	0.130
9	0.474	0.538	0.364	0.043	0.513	0.089
10	0.211	1.000	0.436	0.052	0.328	0.113
11	0.400	0.471	0.371	0.073	0.618	0.099
12	0.105	0.200	0.453	0.056	0.521	0.051
14	0.368	0.150	0.552	0.066	0.457	0.088
15	0.444	0.263	0.564	0.099	0.602	0.095
16	0.353	0.263	0.266	0.034	0.429	0.055
17	0.150	0.250	0.392	0.029	0.517	0.053
18	0.211	0.389	0.450	0.080	0.479	0.041
19	0.333	0.667	0.383	0.046	0.524	0.049
20	0.900	1.000	--		--	
21	0.368	0.438	0.545	0.089	0.508	0.055
22	0.667	0.421	0.406	0.059	0.502	0.120
23	0.615	0.500	0.284	0.106	0.536	0.095
25	0.368	0.313	0.427	0.044	0.290	0.031
26	0.421	0.368	0.424	0.080	0.498	0.057
28	0.375	0.316	0.398	0.059	0.381	0.035
29	0.316	0.611	0.459	0.043	0.601	0.073
30	0.350	0.421	0.417	0.044	0.376	0.060
31	0.235	0.400	0.396	0.073	0.422	0.064
32	0.900	0.833	--		--	