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# Title: A resurrection experiment finds evidence of both reduced genetic diversity and 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 ability to persist and thrive in agricultural fields. Here, we use a resurrection ecology approach and compare the neutral and adaptive genetic variation 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, suggesting that populations of this species sampled from agricultural fields experience genetic bottleneck and/or founder events through time. We further found that populations of this species exhibit modest increases in herbicide resistance over time and evidence that this increase was due to adaptation and not genetic drift. Our results show that even in light of reduced genetic variation, populations of this noxious weed are capable of adapting to strong selection imparted by herbicide application. 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.

## Significance Statement

Although weedy plant species cause significant worldwide declines in crop production, we have a limited understanding of the evolutionary forces that influence their population dynamics. Here we use a novel "resurrection ecology" experiment and examine how both neutral and adaptive genetic diversity change over a nine-year period in the common morning glory, a noxious agricultural weed. By germinating stored seeds and performing genetic diversity and common garden assays we found consistent and striking declines in diversity through time, along with evidence that resistance to the herbicide glyphosate has increased. Our data indicate that weedy plant species are capable of adapting to agricultural regimes even as they experience genetic bottleneck.

## 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 (1). 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 (2). Examples of adaptation to these scenarios are present in the literature from early cases of crop mimicry (1) to the many recent examples of the evolution of herbicide resistance (2). Given the extreme selection that weedy plants may experience in agricultural fields, a central and unanswered question remains: How have many weed species managed to not only adapt to such environments, but also to thrive in them?

These lapses in our understanding of weed evolution are striking because the population dynamics of agricultural weeds are directly relevant to the global food supply. Agricultural weed infestations reduce world-wide crop production by as much as 10% (3), and it has been estimated that crop losses caused by weeds cost the US agricultural economy as much as 33B per year (4). Clarifying the evolutionary forces that impact agricultural weeds can provide information on the

process of evolution more broadly as well as insight on how weeds survive and thrive in agricultural regimes.

Because humans do not intentionally or directly select upon weeds, they are subject to the same forces influencing evolution in nature—notably, genetic drift, selection, and gene flow (5). The difference between natural systems and human-manipulated landscapes is in both the intensity and predictability of selection. For example, the predominant form of weed control in current farming is through the use of herbicides, which are designed to remove 90% of the weed population (6). Individuals that survive due to either chance or genetic predisposition are founders for the next generation. Since the point of weedy plant control regimes—whether through the use of herbicide or another control technique—is to remove of a large portion of the population, populations that re-colonize are likely to show a pattern of constriction and genetic bottleneck (6). Further, as a result of the bottleneck process weeds could lose rare alleles important to future adaptation (7).

In support of this idea, population genetic surveys have found that weeds tend to exhibit less genetic variation than other groups of plants (8), and there is some evidence that weed populations from cultivated land exhibit both decreased neutral genetic diversity and alterations in quantitative traits compared to wild populations (12). The majority of the work to date, however, has compared populations across space, *i.e.,* from cultivated and non-cultivated areas (9), or "wild" versus "weedy" populations (10). In contrast, a novel approach that can provide direct evidence for evolutionary change through time is by the use of "resurrection ecology" in which ancestor and descendant strains of species are compared. In this type of experiment, seeds or propagules sampled from an earlier time point are germinated after remaining dormant for a number of years and compared to descendant populations sampled from the same location (11). Although resurrection ecology experiments have been used to address key questions on evolutionary constraints in microbial systems (12), such experiments in eukaryotes have thus far used either a limited number of accessions (13) or a limited number of distinct populations (14, 15).

Here we perform a resurrection ecology experiment to examine the population genetics and potential adaptability of *Ipomoea purpurea*, the common morning glory. This species is a noxious agricultural weed of corn, cotton and soy crops in the United States as well as a model plant in ecological genetics (16). It is an opportunistic colonizer of disturbed habitats and is found primarily in agricultural fields in the southeastern and Midwestern US. *I. purpurea* exhibits variability in resistance to glyphosate, which is the main ingredient in the herbicide RoundUp, the most utilized herbicide in agriculture world-wide (17). Previous work has found that an additive genetic basis underlies glyphosate resistance in this species (18). More recently we have uncovered evidence of a mosaic of herbicide resistance across the US, with some populations exhibiting high resistance and others showing high susceptibility post-herbicide application (19). Because seeds of *I. purpurea* 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 evidence of adaptive change in plant size and herbicide resistance traits. We find evidence of both genetic bottleneck and adaptive evolution for a key herbicide resistance trait, indicating that a noxious weed can adapt to the extreme selection imposed by herbicide applications even as genetic diversity decreases. 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 evidence for adaptive evolution.

## Results

**Genetic diversity and differentiation**. We uncovered striking reductions in genetic diversity between sampling years among populations (Table 1), with most measures of diversity significantly reduced in 2012 compared to 2003 (Figure 2). For example, expected heterozygosity was 32% lower in 2012 (W = 51, P = 0.01), allelic richness was 18% lower (W = 52, P = 0.01), the effective number of alleles was 43% lower (W = 51, P = 0.01) and the absolute number of alleles per locus were reduced by 19% in 2012 compared to 2003 (W = 50, P = 0.01). Interestingly, the observed heterozygosity was 27% higher, on average, in 2012 compared to 2003 (W = 4, P = 0.005). This difference is likely due to the low observed compared to expected heterozygosity of the 2003 cohort, *i.e.,* the fixation index ($F = H_e\text{-}H_o/H_e$) was higher in 2003 versus 2012 ($F_{2003} = 0.53\pm0.04$ vs. $F_{2012} = 0.17\pm0.05$, respectively). Although this difference could be due to selection against heterozygotes in 2003, it is more likely indicative of differences in the mating system between sampling years of this mixed-mating, hermaphroditic species. Populations were sampled during a longer window of time in 2003 than in 2012 (10/10-11/3 in 2003 vs 10/15-10/20 in 2012); however, at least five of the 10 populations were sampled during the same temporal window (10/10-10/20 both years), and these populations still exhibit differences in F values ($F_{2003} = 0.46\pm0.08$ vs. $F_{2012} = 0.11\pm0.03$). We do not have information regarding pollinator abundance or any other reason to expect differences in the mating system between years and thus cannot explain this pattern. HWE tests, consequently, indicated that more loci were not in HWE equilibrium within populations in 2003 (86 out of 150 loci × population combinations), compared to 2012 (24 out of 150).

Alternatively, increased heterozygosity in bottlenecked versus source populations could be due to random drift leading to an increase in the frequency of formally rare alleles (20); such changes can subsequently lead to higher observed heterozygosity in bottlenecked populations relative to their source populations (21). We thus examined changes in the patterns of allelic diversity by investigating the number of alleles, the number of rare alleles (<10% frequency) and the frequency of rare alleles present in 2003 and 2012. At the species level (*i.e.,* across all populations), we found no evidence for a reduction in the total number of alleles from 2003 to 2012 (42 versus 44 alleles in each year, respectively)—unexpectedly, we found fewer rare alleles in 2003 than 2012 (10 vs 17). Only four of the rare alleles present in 2003 were likewise present in 2012, and their frequency was not dramatically increased as would be expected if rare allele frequency changes were responsible for the higher observed heterozygosity in 2012. When

155 examining the number of alleles per population, however, we found that the total number of
156 alleles was reduced in eight of 10 populations, by as much as 12-40% across populations. Two of
157 the ten populations (populations 26 and 28) exhibited gains of low frequency alleles (between 4-
158 5 new alleles present in 2012 at frequencies of <10%). Thus, 8 of the 10 populations show
159 reductions in diversity over time likely due to random genetic drift, whereas two of the
160 populations exhibit an increase in the number of alleles, putatively due to migration from other
161 sources. Finally, bottleneck tests using either the signed, Wilcoxon or standard differences test
162 for an infinite allele model indicated that between 2-5 populations, depending on the test, from
163 the 2012 seed cohort experienced bottleneck (Table S2). We did not perform bottleneck tests
164 using the 2003 cohort since such tests are sensitive to deviations from HWE.

165 We next estimated the effective number of individuals from each sampling year using
166 expected heterozygosity and the equation $H_e = 4N_e\mu$ (22) with a mutation rate, $\mu$, of $10^{-3}$ (23).
167 We found the estimated number of individuals from the 2003 populations was significantly
168 higher, on average, compared to that of the 2012 populations ($N_{e,\,2003} = 85$, $N_{e,\,2012} = 58$; W = 67,
169 P = 0.005). Furthermore, we found no significant difference between our census sample size
170 from the 2012 populations and the estimated effective number of individuals from that sampling
171 year (Population size average from census = 70 individuals; W = 36.5, P = 0.32). While the
172 difference in estimated number of individuals between sampling years indicated that most
173 populations experienced reductions in size (reductions ranging from 20-55 individuals fewer in
174 2012), populations 26 and 28 both exhibited an estimated gain of 20 individuals.

175 In line with lower diversity of the majority of populations, we found significant genetic
176 differentiation between individuals sampled from different collection years (AMOVA year
177 effect, $F_{RT} = 0.306$, P = 0.001, Table S3), and evidence that individuals sampled as seed in 2003
178 were more similar to one another than to individuals sampled as seed from the same location in
179 2012 (Figure 3), *i.e.,* no individual assigned to 2003 was likewise assigned to 2012. Populations
180 sampled in 2003 exhibited lower estimates of genetic differentiation than the same populations
181 collected in 2012 ($F_{ST2003} = 0.113$, $P < 0.001$; $F_{ST2012} = 0.309$, $P < 0.001$), supporting the idea that
182 the populations sampled in 2003 experienced a bottleneck leading to greater genetic divergence
183 in 2012.

184 **Trait divergence.** We examined resistance traits (survival and biomass post-herbicide
185 application), and plant size traits (height and number of leaves) from two separate experiments—
186 one screening for resistance and another for plant growth—to determine if there was evidence of
187 adaptive trait differentiation between sampling years. Our mixed-effects analyses of variance
188 uncovered a significant year effect for biomass remaining after herbicide application ($F_{1,\,3595} =$
189 4.72, P = 0.03; Table S4). On average across all populations, the biomass remaining post-spray
190 of the 2012 cohort was slightly greater than that of the 2003 cohort (62% vs. 57% in 2012 and
191 2003, respectively) suggesting moderate increases in resistance across populations sampled in
192 2012 (see Table S5 for averages (±SE) among all populations). Likewise, a higher percentage of
193 individuals sampled in 2012 survived herbicide application compared to those sampled from
194 2003 (49% vs 42%), but this difference was not significant ($F_{1,\,5365} = 2.58$, P = 0.11; Table S4).

As in previous work (19), we identified significant population effects for both measures of resistance (Table S4), indicating that populations vary across the landscape for their relative level of herbicide resistance. Here, however, we also find population by year effects in each analysis, indicating that populations differ in their level of resistance across years (Survival, $\chi^2 = 23.74$, $P < 0.001$; Biomass, $\chi^2 = 7.92$, $P = 0.005$; Table S4). One population's survival increased by 79% compared to 2003, indicating that some populations may respond more readily with increased resistance than others. In general, three populations sampled from TN that were highly resistant in 2012 (19) were similarly resistant in 2003 (Figure 1 A & B, shown at 2X field rate of RoundUp). The majority of the significant increases identified in the 2012 cohort compared to the 2003 cohort were located in NC and SC (Figure 1 A & B)—while five populations from the 2012 cohort of this region exhibit resistance values significantly greater than the species-wide average (56% survival at 2X the field rate of RoundUp; (19)), the 2003 cohorts of these populations exhibited only ~14% survival at 2X field rate.

Although we found evidence for differences in resistance traits between years, we found no significant differences in the size of the plants sampled from 2012 compared to 2003. The height of the plants and the number of leaves were both approximately the same, on average, between sampling years (105 vs 102 cm and 8.8 vs 8.8 leaves, 2003 vs 2012, respectively; see Table S4).

**Adaptive evolution.** We next conducted a $P_{CT}$-$F_{CT}$ (*i.e*, Pseudo $Q_{ST}$-$F_{ST}$; (24)) analysis modified from Duncan and Rausher (25) to determine if the moderate increase in resistance in 2012 (*i.e.*, increased biomass remaining after herbicide) was consistent with neutral divergence or due to adaptive evolution over time. We performed the analysis for biomass after herbicide application since this was the only trait that exhibited a significant effect of year. This analysis is a hierarchical extension to typical $Q_{ST}$-$F_{ST}$ analyses in that the variance components of year and population nested within year are included, and it is considered a "Pseudo $Q_{ST}$" since we are utilizing population variation rather than a half- or full-sib design. If $P_{CT} > F_{CT}$, positive/directional selection would be inferred as the reason for trait divergence among years whereas the data are consistent with genetic drift if $P_{CT} = F_{CT}$. In contrast, if $P_{CT} < F_{CT}$, we would conclude that the trait is subject to stabilizing or uniform selection across years. We found that the $P_{CT}$ value for biomass remaining after herbicide application was greater than and strongly differentiated from the $F_{CT}$ value (Figure 4), *e.g.*, the distribution of bootstrap values of $F_{CT}$ ($F_{CT} = 0.30$, 95% CI: 0.24-0.35) did not overlap the $P_{CT}$ distribution ($P_{CT} = 0.71$, 95% CI: 0.37-0.88). Thus, the increase in resistance identified between sampling cohorts is consistent with a scenario of adaptive evolution rather than due to neutral processes such as drift.

## Discussion

Despite the ubiquity and persistence of weedy plant populations, there are few examinations of how their neutral and adaptive genetic diversity may change over time. Here we use a resurrection ecology experiment to show that populations of weedy *I. purpurea* sampled from crop fields concomitantly lose genetic diversity and show signs of adaptive evolution in an

herbicide resistance trait. Our experiments yielded three novel findings. First, we found that seed progenies from populations sampled in 2012 exhibited lower genetic diversity and higher genetic differentiation than seed progenies sampled from the same fields and locations in 2003. Second, in addition to finding population variation for resistance traits, we found that a key resistance trait—the amount of biomass maintained following herbicide application—has increased from 2003 to 2012. Third, a hierarchical examination of adaptive and neutral genetic variation, which included the variance due to collection year, showed a pattern of $P_{CT} > F_{CT}$ for biomass remaining after herbicide, suggesting that the increase in resistance we detected between 2003 and 2012 was the result of adaptive evolution and not genetic drift.

Previous examinations of agricultural weed populations compared to wild populations have either failed to uncover substantial reductions in genome-wide diversity (10) or have presented largely circumstantial evidence for bottlenecks (i.e., comparisons between species; (8)). The significant loss in diversity that we uncovered across populations of *I. purpurea* sampled from agricultural fields argues for a bottleneck that was either very strong or occurred with high frequency, or both. Although there are no studies, to our knowledge, that examine the temporal genetics of agricultural weed populations for comparison, it is of note that the average loss of allelic richness that we identified across populations (on average 15% lower between cohorts) is similar in magnitude to that of introduced, colonizing species (18% loss compared to native populations (26). Furthermore, using expected heterozygosity estimates from each sampling year, we find that the estimated population sizes have reduced between 2003 and 2012, with the majority of the populations losing reproductive individuals. While we did not take population census data in 2003 for comparison, we find that the estimated population size in 2012 is not significantly different from the census size, suggesting that our estimated population sizes are decent approximations of the true sample size. While the majority of the populations exhibited loss of alleles between sampling years, two populations—#26 and #28—exhibited gains of low frequency alleles, and the estimated sample size of these two populations likewise increased relative to other populations. The increased diversity of these populations likely were from dormant seeds, or, alternatively, due to migration from another population. Emergence of seed stored in the seed bank is incredibly likely—this species can produce a large number of seeds in field conditions (between 3,000-10,000 per individual), and these heavy, gravity dispersed seeds can remain dormant for ~20 years in the soil (27).

Recent work provides an interesting contrast between the phenotypic and neutral genetic variation spatially distributed within this system—while neutral genetic differentiation among 44 *I. purpurea* populations is low (*i.e.,* $F_{ST} = 0.127$), populations are significantly differentiated for herbicide resistance across the landscape, with some populations exhibiting high and others very low resistance (19). Our screen of adaptive variation in 26 of these temporally sampled populations shows that, in addition to a mosaic of resistance across the landscape, the level of resistance in this species has increased over time. This finding is especially interesting in light of the reduced neutral genetic variation that we identified and, alternatively, evidence of potential migrants in the 2012 cohort—reductions in diversity as well as influx of presumably non-adapted variation would either act to impede or to counteract adaptation. These forces, along with recent work showing a severe fitness penalty of herbicide resistance in this species (28) likely explain

why the average increase in resistance that we identified was modest—perhaps the populations that maintained resistance between sampling years (TN populations) or those that exhibit large increases in resistance (NC/SC populations) were less influenced by susceptible migrants germinating from the seed bank and/or costs of resistance than other populations. Interestingly, there was no evidence that plants were different in size between the years, indicating that the increased resistance we detected is not due to plants simply being larger in the 2012 cohort and thus better able to withstand herbicide application.

Further, we found that the temporal increase in resistance is inconsistent solely with the action of genetic drift. Our $P_{CT}$-$F_{CT}$ comparisons indicate that the variation between years in biomass remaining after herbicide application was significantly greater than variation due to neutral processes, which suggests resistance has increased due to the action of selection and subsequent adaptation. Although these data are compelling, they must be interpreted cautiously. $P_{CT}$ estimates are sensitive to environmental and non-additive genetic effects (29); because we are assessing population-level variation and did not use a half- or full-sib design we are not able to decouple additive genetic variation from maternal and paternal effects as well as other potential environmental influences (24). However, we have previously identified an additive genetic basis underlying glyphosate resistance in one population of this species (18), and, further, positive selection for increased resistance in the field (18). Thus, although the $P_{CT}$ estimate provided here includes many sources of variation, the presence of genetic variation and evidence for selection on this variation broadly supports a scenario of adaptive evolution. Further, we know that selection *via* glyphosate application was relatively consistent across these sampled locations—we have historical record for six of 10 years (Table S1) which shows that these locations were used for corn and soy crops, both of which make use of herbicides for weed control. Given that ~98% of soy planted since 2004 has been RoundUp Ready, and that on average over 50% of corn crops are also RR (30), it is highly likely that our sampling locations have experienced glyphosate application on a regular or somewhat consistent basis. Thus, in sum, all the pre-requisites for the evolution of herbicide resistance by adaptation are present in this system, including evidence of a consistently applied selective agent. That we identify an increase in resistance between two temporally distinct samples provides strong evidence that glyphosate resistance results from adaptation to the agricultural regime. Identification of the genetic basis of resistance in this system, and an assessment of how alleles associated with resistance change over time will decisively test our hypothesis that selection from the use of this herbicide is leading to adaptation.

Conclusions—Weedy plant species found in agricultural fields experience strong selection and thus are hypothesized to be either plastic, capable of adaptation, or saved from extinction through gene flow (31, 32). By using a resurrection ecology framework, we provide evidence that even though genetic variation is lost from the system, populations show signs of adaptation to herbicide application. While previous work indicates that the majority of the gene flow across southeastern populations occurred prior to the wide-spread adoption and use of glyphosate, suggesting that resistance evolution is due to selection on standing or novel variation within populations, that we identified evidence of potential migrants into the 2012 gene pool does not allow us to rule out the hypothesis that resistance is introduced from outside sources.

Further, while we find evidence of increased resistance, we also show that the absolute change between years was not drastic; large resistance gains were limited to particular populations. These data suggest heightened measures should be taken to reduce the likelihood that seeds are accidentally moved between crop fields through farm machinery or through contaminated seed lots. Finally, we do not have evidence that the lower genome-wide diversity identified across populations is due the application of glyphosate *per se*—other herbicides with different mechanisms of action are often applied in corn 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, as found in Nevo, 2012 (33). The results shown here suggest that this weed, while being a 'general purpose genotype'(1, 34) 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.

## Materials and Methods

**Population sampling.** Population locations and sampling strategies for 44 *I. purpurea* populations were previously described in Kuester *et al* (19). Twenty-six of these populations were sampled in 2003 and resampled in 2012 (see Figure 1). In each year, we collected replicate seeds from between 6-30 maternal individuals at least 1 m apart from one another along a linear transect. We estimated population size in the 2012 sampling year by counting the numbers of individuals down a linear transect. We used replicate seeds from maternal individuals sampled in both 2003 and 2012 from the same locations to perform three separate experiments: 1) a screen of neutral genetic diversity, 2) an assessment of herbicide resistance, and 3) a comparison of growth and size traits.

Of the 26 populations that were sampled both years, we randomly chose 10 to examine potential changes in genetic diversity between 2003 and 2012. One seed from an average of 18 maternal lines per population per sampling year (355 individuals total) were germinated and cotyledons were used for DNA isolation using a CTAB method modified from Stokes et al. 2009 (see Kuester *et al.* (19) for details).

To assay herbicide resistance among populations and between sampling years, we planted two replicate greenhouse experiments of all 26 populations at the University of Georgia Plant Biology Greenhouses (Athens, GA). One seed from 10 maternal lines per population per sampling year were scarified and planted in pine bark soil in SC10 super conetainers (Stuewe and Sons, Tangent, OR) in six experimental treatments, described below. This design was replicated in its entirety in another greenhouse for a total of 20 seeds per population within each treatment and thus an overall total of 5381 experimental individuals. Plants were randomly assigned to racks that were then randomly assigned to flow trays (4 racks per flow tray). Conetainers were watered daily and flow trays were filled with water to prevent desiccation. Germination was slightly higher in 2003 compared to 2012 samples (87% and 84% in 2003 and 2012, respectively, $\chi^2_1 = 12.27$, $P < 0.001$) and ranged from 50-98% across populations. While mutations could have accumulated in the stored seeds sampled from 2003, that our germination rates were moderately higher in the 2003 versus the 2012 cohort suggests that our result of strikingly lower allelic variation in the 2012 sample (see Results) was not driven primarily by seed storage of the 2003 cohort.

361 Plants were sprayed with RoundUp PowerMax (Monsanto, St Louis, MO) 22 days after
362 planting at rates around the recommended field rate (1.54 kg ai/ha) of 0, 0.21, 0.42, 0.84, 1.70
363 and 3.40 kg a.i./ha (the 0 kg a.i./ha control treatment was sprayed with water) using a hand-held,
364 $CO_2$ pressurized sprayer (R & D Sprayers, Opelousas, LA). We sprayed plants at a speed of 187
365 liters/ ha at 30 psi with a stride pace of 90 paces per minute at 1.5 meters above the plants. Three
366 weeks after glyphosate application we scored survival of each plant. Plants were harvested, dried
367 at 72°C for 48 hours and measured for total above ground biomass. Biomass values were
368 adjusted to the non-sprayed controls by dividing each individual by the average biomass of its
369 population grown in the non-spray control treatment following standard protocols (35).

370 Finally, to examine the potential for growth and size changes between 2003 and 2012,
371 seeds from 20 individuals from each of fifteen randomly sampled populations from each year
372 were nicked and planted in 6 inch pots in the University of Michigan greenhouses at Matthaei
373 Botanical Gardens in May 2014. Two replicate experiments were planted two weeks apart (351
374 and 375 individuals in each of the two experimental replicates), for a total of 726 experimental
375 individuals. We measured plant height and the number of leaves after plants had grown 4 weeks
376 in standard greenhouse conditions.

377 **SSR genotyping and scoring errors.** Details on multiplexing SSR markers and scoring
378 procedures can be found in Kuester et al (19). Briefly, 15 polymorphic microsatellite loci were
379 used to examine genetic diversity across populations and sampling years, and all individuals
380 were scored by hand. We randomly double-scored 100 individuals across loci to check accuracy
381 of multi-locus genotypes, and found very few scoring errors and no large allele drop outs or
382 scoring errors due to stutter in any of the locus by population by year combinations. We also
383 examined the influence of null alleles on genetic differentiation and found little evidence that
384 potential null alleles changed our $F_{CT}$ values. Details of these analyses are presented in the SI
385 Materials and Methods.

386 **Temporal genetic differentiation and diversity**. We examined the potential that seeds sampled
387 across collection years were genetically differentiated from one another in two ways. First, we
388 estimated genetic differentiation between years using hierarchical AMOVA in GenAlEx v. 6.5
389 (36). We also performed individual assignment (37, 38) of individuals to sampling year using
390 GeneClass2 (39). For individual assignment, the inability to assign individuals to a specific
391 sampling year would indicate that individuals sampled in 2012 had not diverged in allelic
392 composition compared to the individuals sampled in 2003. We used the Bayesian method
393 described by Baudouin and Lebrun (40) as a criterion for computation, and individual
394 assignment was performed using the leave-one-out procedure (41), where the genotype to be
395 assigned was not included in the population from which it was sampled. We report the –log
396 likelihood of being assigned in each sampled year, by plotting the –log likelihood value of
397 individual assignment to 2003 sample year against the - log likelihood of being assigned to the
398 2012 sampling year. Lack of temporal change across sampling years would be indicated by
399 overlap of individuals sampled from each year. We calculated expected and observed
400 heterozygosity ($H_e$ and $H_o$), the number of alleles (Na) and the number of effective alleles (Ne)
401 using GenalEx v 6.5 (36) and gene diversity (GD) and allelic richness (AR) using FSTAT v.
402 2.9.3.2 (42) and determined if there were reductions in diversity estimates between 2003 and
403 2012 using Wilcoxon matched pairs rank sum tests (43). Finally, we examined the possibility
404 that populations experienced genetic bottleneck using the program BOTTLENECK (39). Three
405 significance tests described by Cornuet and Luikart (38) and (44) signed rank, standard

differences and Wilcoxon test) were assessed under an infinite allele model of microsatellite evolution.

**Resistance screen and growth traits.** We examined whether populations and sampling years varied for resistance or growth and size traits using univariate mixed-model analyses of variance. For the resistance screen, we used the lmer option of the lme4 package in R (45) for our models in which replicate greenhouse experiment, herbicide treatment, collection year, and population were the independent variables with standardized biomass and survival as the dependent variables. Survival was modeled as a binary character (0/1). We included interactions between population and collection year as well as population, collection year and treatment. Population and its interaction terms were considered random effects in the model whereas all other effects were fixed. We previously identified a significant population effect from the 2012 cohort for survival post-herbicide application, which indicated that populations vary in their respective level of resistance (19). Here we are specifically interested in the year term as well as interaction terms including the year effect, which would indicate that resistance varies between sampling years and/or that populations vary in their level of resistance between years. An F-test was used to determine the significance of fixed effects, and the significance of each random effect in the model was determined using a likelihood ratio test (LRT) in which the full model was compared to a reduced model with the effect of interest removed. The *P*-value was determined using a $\chi^2$ test with one degree of freedom.

Finally, to determine if populations or individuals from different sampling years varied for growth traits, we ran univariate analysis of variance with experimental replicate, sampling year, population and the population x year interaction term as independent variables with height and the number of leaves as the independent variables using the lmer option of the lme4 package (45) in R v 3.1.1 (46). Experimental replicate and sampling year were considered fixed effects in each model with population and its interactions considered random effects. The significance of each effect in the model was examined as in the resistance assay above. Height and the number of leaves were modeled with Poisson distributions.

In all models, we examined the normality of all dependent variables with the Shapiro-Wilk test and by visual inspection of quantile-quantile (q-q) plot. Biomass following herbicide application was square root transformed to improve normality of the residuals.

**Adaptive trait differentiation**. We next employed a hierarchical $P_{CT}$ (Pseudo-$Q_{CT}$)-$F_{CT}$ comparison modified from Duncan and Rausher (25) to determine if there was evidence of adaptive evolution among sampling years. In the analyses here, populations are nested within sampling years and there are thus two hierarchical levels—among populations and among sampling years. To our knowledge, few studies use nested $P_{CT}$-$F_{CT}$ comparison in a temporal context (but see (11)). For the resistance trait (biomass remaining after herbicide application), we calculated the summary statistic $P_{CT}$ using the method developed by Whitlock and Gilbert (47), which describes the amount of variation for a morphological trait between sampling years:

$$P_{CT} = V_C /(V_C+V_P+2V_I),$$

where $V_C$, $V_P$ and $V_I$ are the components of genetic variation between sampling years, among populations, and among individuals within populations, respectively. We compare $P_{CT}$ to $F_{CT}$

using bootstrap sampling. To generate a bootstrap sample from each of the sampling years, we randomly sampled population from a year. We next randomly drew n individuals without replacement, where n equals the number of individuals originally sampled per population. This sampling procedure was performed for each sampling year (2003 and 2012 samples). We performed 1000 bootstrap replicates. $P_{CT}$ or $F_{CT}$ were calculated from a nested ANOVA or AMOVA (population nested within year) for each bootstrap sample. Because we could not estimate heritability from our data, we directly compared $P_{CT}$ to $F_{CT}$ at $h^2 = 1$, at which $V_I$ was set equal to the within-population portion of variation from the ANOVA. Since it is very unlikely that all of the within-population variance explains the fraction due to the additive genetic variance, we compared $P_{CT}$ to $F_{CT}$ over a range wherein we scaled the $V_I$ by a factor from 0 to 1. Estimates were similar across this range and are not reported; further, presenting the results at $h^2 = 1$ produces a smaller $Q_{CT}$ and is thus more conservative. We present 95% confidence intervals of bootstrapped estimates for $P_{CT}$ and $F_{CT}$ as evidence of selection.

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## 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 gene diversity (GD).

| | Na | | Ne | | Ho | | He | | AR | | GD | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | 0.31 | 0.23 |
| 8 | 2.33 | 1.67 | 1.76 | 1.31 | 0.17 | 0.18 | 0.38 | 0.17 | 2.16 | 1.48 | 0.41 | 0.17 |
| 10 | 2.20 | 1.93 | 1.65 | 1.29 | 0.13 | 0.15 | 0.33 | 0.18 | 1.97 | 1.59 | 0.35 | 0.19 |
| 18 | 2.40 | 1.47 | 1.76 | 1.27 | 0.13 | 0.15 | 0.36 | 0.16 | 2.05 | 1.41 | 0.38 | 0.17 |
| 21 | 2.53 | 1.40 | 1.90 | 1.14 | 0.14 | 0.12 | 0.41 | 0.18 | 2.29 | 1.53 | 0.43 | 0.20 |
| 23 | 2.53 | 1.93 | 1.84 | 1.46 | 0.16 | 0.24 | 0.39 | 0.27 | 2.18 | 1.75 | 0.40 | 0.29 |
| 26 | 1.80 | 2.13 | 1.39 | 1.61 | 0.18 | 0.24 | 0.23 | 0.31 | 1.61 | 1.87 | 0.24 | 0.32 |
| 28 | 2.13 | 2.40 | 1.40 | 1.55 | 0.15 | 0.28 | 0.23 | 0.31 | 1.71 | 1.94 | 0.24 | 0.32 |
| 30 | 2.27 | 1.93 | 1.86 | 1.48 | 0.19 | 0.27 | 0.40 | 0.28 | 2.14 | 1.73 | 0.42 | 0.29 |
| 32 | 2.40 | 1.80 | 1.73 | 1.43 | 0.14 | 0.23 | 0.37 | 0.24 | 2.14 | 1.66 | 0.39 | 0.25 |

**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 and growth trait measurements). The percent survival following 3.4 kg ai/ha of RoundUp is indicated in color. Sites were sampled at least 5 km apart.

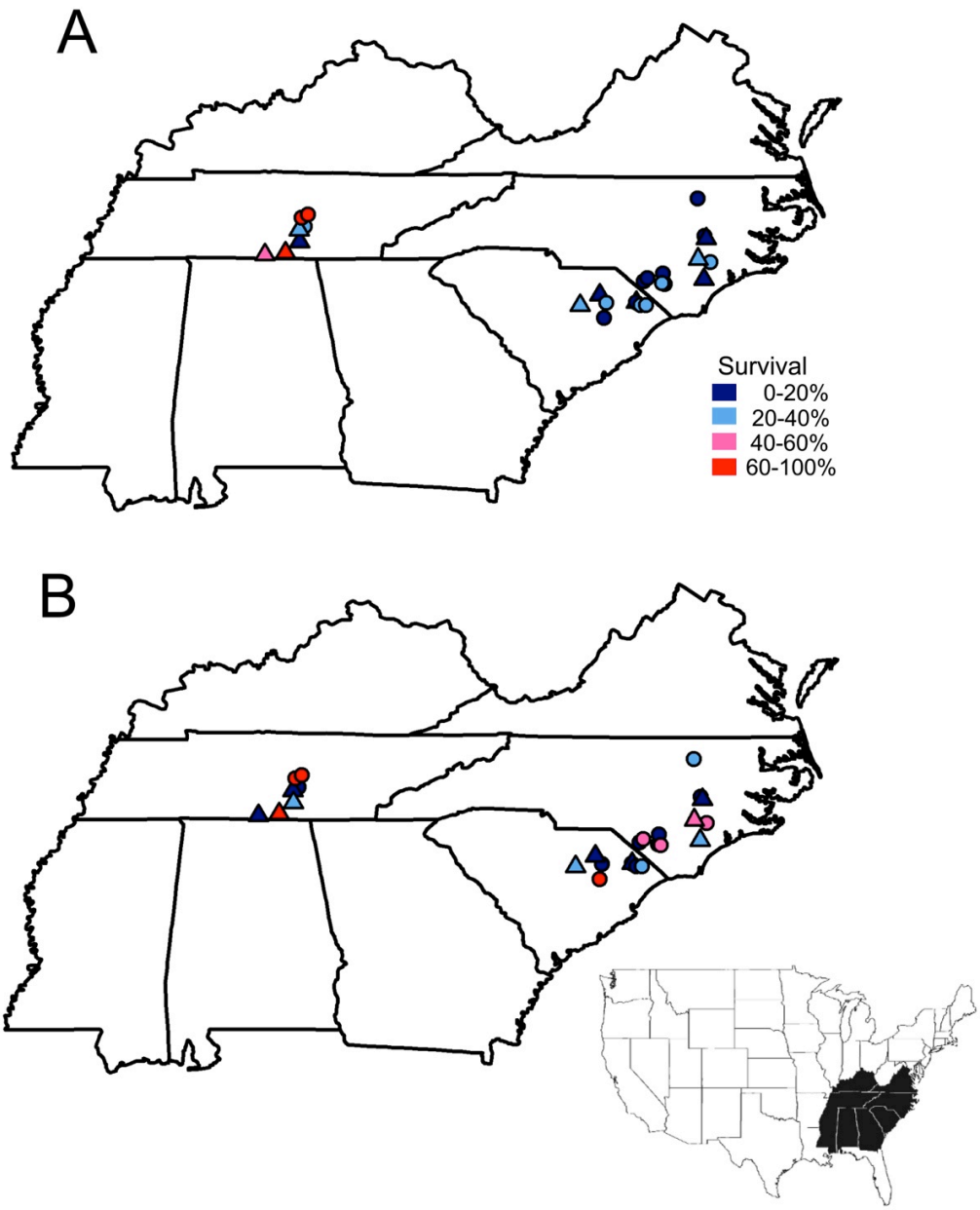


Figure 2. Genetic diversity indices compared between sampling years (2003 and 2012). Shown are (A) number of alleles (Na), (B) effective number of alleles (Ne), (C) observed heterozygosity (Ho), (D) expected heterozygosity (He), (E) allelic richness (AR), and (F) gene diversity (GD). Mean values for each year are depicted plus or minus one standard error.

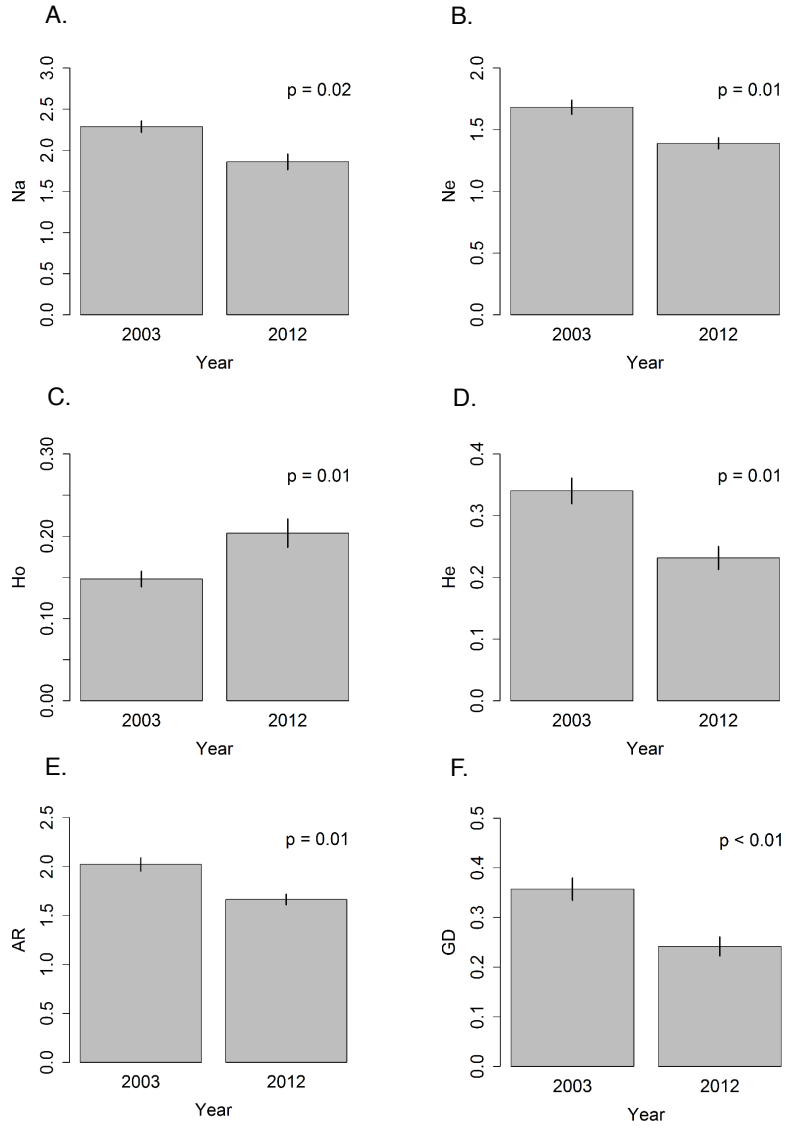


Figure 3. Scatter plots of log likelihood values from assignment tests of individual *I. purpurea* plants sampled in 2003 and 2012 based on genotypic data at 15 microsatellite loci. A higher position relative to the y-axis indicates a higher likelihood of being from 2012 pool of individuals and a higher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

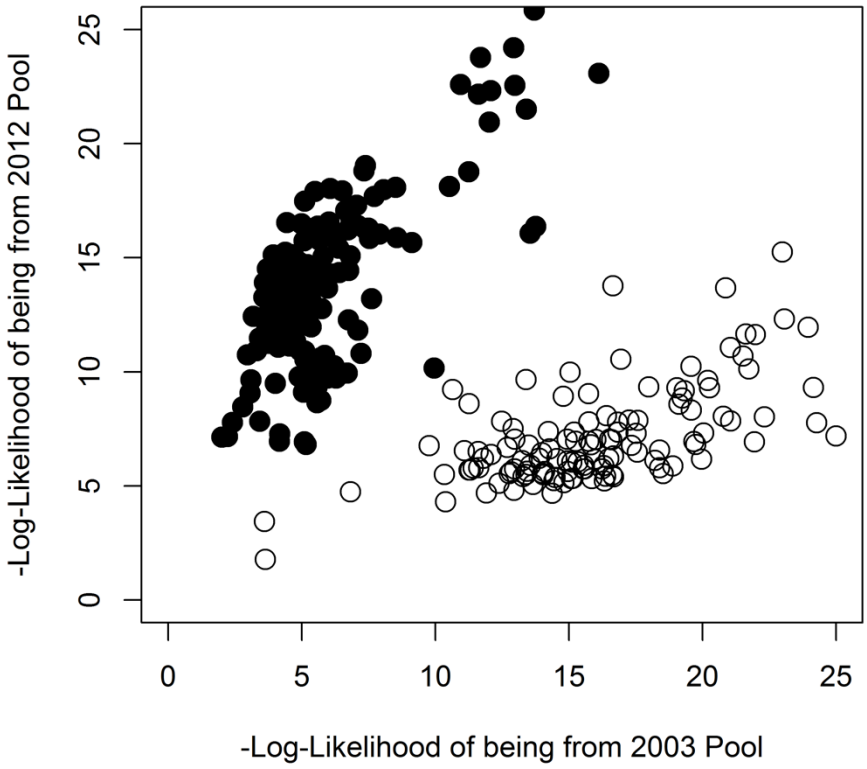
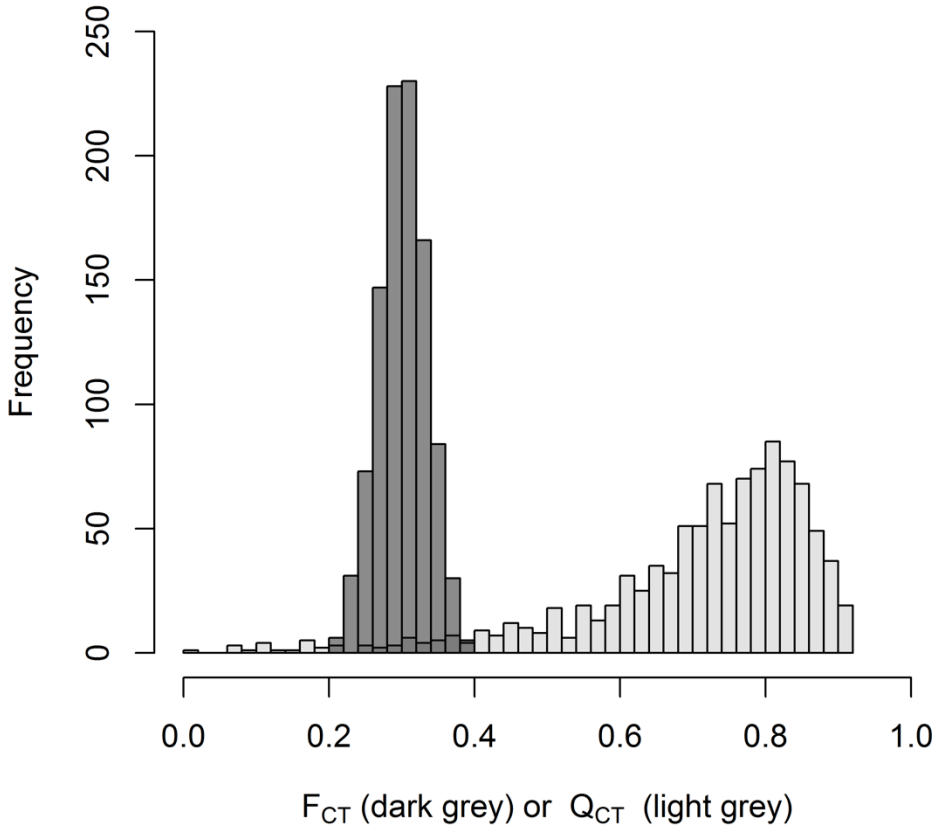


Figure 4. $P_{CT}$ (light grey) vs $F_{CT}$ (dark grey) analysis of biomass remaining after herbicide application, estimated at $h^2 = 1$. Frequency indicates the number of bootstrap runs that returned $P_{CT}$ and $F_{CT}$ estimates.



**Supporting Information**

Materials and Methods

*Scoring errors*—We assessed scoring errors per locus x population combination using MicroChecker (Van Oosterhout et al. 2004). Null alleles were detected in 13 of 15 loci (5-40% of populations, mean = 25%). We found no significant difference in genetic structure (uncorrected $F_{CT}$: 0.33-0.46, corrected $F_{CT}$: 0.31-0.43) after adjusting for the presence of potential null alleles. 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.

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*—Deviations from Hardy-Weinberg equilibrium (HWE) were tested using GenePop v 3.3 (Raymond and Rousset 1995). Of 150 locus x population tests, 86 locus x population pairs from 2003 and 24 pairs from 2012 deviated from HWE, with ranges of loci deviating per population from 5-11 and 0-5 from 2003 and 2012, respectively. We similarly found much higher values of $F_{IJ}$ in 2003 compared to 2012 (average $F_{IJ\ 2003} = 0.529$, average $F_{IJ\ 2012} = 0.165$).

*Loci under selection*—Loci under selection were identified using BayeScan (Foll and Gaggiotti 2008), which uses a multinomial-Dirichlet model using inter-population differences in allele frequency patterns (Foll et al. 2010). We used 5000 iterations and default model parameters for loci assessment. We detected 5 outlier loci with signature of diversifying selection across populations (IP31, IP8, IP18, IP1, IP26). When we removed these five loci from our estimate of $F_{CT}$, we found little difference from $F_{CT}$ estimation with all loci (95% C.I. $F_{CT\ 15\ loci}$ : 0.232-0.364, 95% C.I.$F_{CT\ 10\ loci}$: 0.245-0.372 ), which did not impact our interpretation of $Q_{CT}$-$F_{CT}$ comparison for biomass data (95% C.I. $Q_{CT\ Biomass}$: 0.416-0.941).

*Linkage disequilibrium*—We tested the presence of linkage disequilibrium between locus pairs for each population x sampling year combination using GenePop v 3.3 (Raymond and Rousset 1995). 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 in 2012 ($P < 0.05$). The majority of locus pairs in disequilibrium were from population 26 (25 significant pairs).

*Scoring accuracy*— We found the scoring error rates per locus after re-scoring 200 individuals: 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%).

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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**. Test for Cornuet and Luikart's (1996) bottleneck detection using an infinite allele model of microsatellite evolution. Shown are p-values from three tests described by Cornuet and Luikart (1996) and Luikart et al. (1997) for each population sampled in 2012.

| Population | Signed test | Standard Differences | Wilcoxon Test |
|---|---|---|---|
| 2 | 0.502 | 0.267 | 0.313 |
| 8 | 0.247 | 0.163 | 0.230 |
| 10 | 0.541 | 0.333 | 0.765 |
| 18 | 0.025 | 0.036 | 0.027 |
| 21 | 0.557 | 0.232 | 0.371 |
| 23 | 0.170 | 0.047 | 0.095 |
| 26 | 0.389 | 0.050 | 0.122 |
| 28 | 0.296 | 0.221 | 0.249 |
| 32 | 0.126 | 0.024 | 0.012 |
| 30 | 0.035 | 0.017 | 0.032 |

**Table S3**. Analysis of Molecular Variance (AMOVA) of neutral genetic data. Shown 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.

| Source | df | F-Statistics | Value | P |
|---|---|---|---|---|
| Year | 1 | $F_{RT}$ | 0.306 | 0.001 |
| Population(Year) | 18 | $F_{SR}$ | 0.214 | 0.001 |
| Individual (Population) | 335 | $F_{ST}$ | 0.454 | 0.001 |
| Individual (Total) | 355 | $F_{IS}$ | 0.514 | 0.001 |
| Total | 709 | $F_{IT}$ | 0.735 | 0.001 |

**Table S4**. Generalized linear mixed effects model of (A) resistance and (B) size traits 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 was modeled with a binomial distribution, biomass after herbicide fit a gaussian distribution, and plant height and number of leaves were best modeled with a poisson distribution. Biomass was square-root transformed prior to analysis. For each model, we show the degrees of freedom (Df), sums of squares (SS), mean square error (MSE), F or $\chi 2$ statistic and associated P value.

A.

| | Survival | | | | | Biomass | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Fixed Effects | Df | SS | MSE | F | P | Df | SS | MSE | F | P |
| Replicate | 1 | 14.460 | 14.459 | 14.459 | <0.001 | 1 | 0.625 | 0.625 | 12.575 | <0.001 |
| Treatment | 5 | 773.710 | 154.742 | 154.742 | <0.001 | 5 | 47.495 | 9.499 | 190.978 | <0.001 |
| Year | 1 | 2.580 | 2.578 | 2.578 | 0.108 | 1 | 0.235 | 0.235 | 4.724 | 0.030 |
| Year x Treatment | 5 | 0.460 | 0.092 | 0.092 | 0.994 | 5 | 0.213 | 0.043 | 0.855 | 0.511 |
| Random Effects | | | | $X^2$ | | | | | $X^2$ | |
| Population | 1 | | | 19.179 | <0.001 | 1 | | | 4.972 | 0.026 |
| Population x Year | 1 | | | 23.747 | <0.001 | 1 | | | 7.921 | 0.005 |
| Population x Treatment | 1 | | | 3.701 | 0.054 | 1 | | | <0.001 | 1.000 |
| Population x Treatment x Year | 1 | | | <0.001 | 1.000 | 1 | | | <0.001 | 1.000 |
| Residual DF | 5365 | | | | | 3595 | | | | |

B.

| | Height | | | | | Number of Leaves | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Fixed Effects | Df | SS | MSE | F | P | Df | SS | MSE | F | P |
| Replicate | 1 | 449.7 | 449.7 | 449.7016 | <0.001 | 1 | 4.458 | 4.458 | 4.458 | 0.035 |
| Year | 1 | 0.63 | 0.63 | 0.6269 | 0.429 | 1 | 0.0304 | 0.0304 | 0.0304 | 0.862 |
| Random Effects | | | | $X^2$ | | | | | $X^2$ | |
| Population | 1 | | | 0.6457 | 0.4217 | 1 | | | 2.142 | 0.1433 |
| Population x Year | 1 | | | 1496.4 | <0.001 | 1 | | | 0 | 1 |
| Residual | 706 | | | | | 706 | | | | |

**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.

A. Resistance Traits

| | 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 | -- | | 0.144 | 0.133 |

B. Growth Traits

| | Height | | | | Number of Leaves | | | |
|---|---|---|---|---|---|---|---|---|
| | 2003 | | 2012 | | 2003 | | 2012 | |
| PopID | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 1 | 101.72 | 6.67 | 100.16 | 4.11 | 8.10 | 0.51 | 8.40 | 0.39 |
| 2 | 108.95 | 3.27 | 110.25 | 4.01 | 9.40 | 0.32 | 9.26 | 0.37 |
| 4 | 116.29 | 3.90 | 113.94 | 4.73 | 9.57 | 0.38 | 9.27 | 0.50 |
| 5 | 106.93 | 5.12 | 117.77 | 6.42 | 9.00 | 0.57 | 10.41 | 0.91 |
| 8 | 97.62 | 9.90 | 100.91 | 7.32 | 8.86 | 0.95 | 8.75 | 0.62 |
| 9 | -- | | 98.27 | 7.82 | 8.20 | 2.15 | 8.53 | 0.73 |
| 10 | -- | | 98.13 | 7.75 | 7.71 | 1.39 | 8.76 | 0.90 |
| 12 | 113.69 | 12.12 | 125.62 | 9.41 | 9.07 | 0.37 | 8.31 | 1.46 |
| 14 | 96.47 | 11.56 | 78.16 | 6.66 | 8.67 | 0.94 | 8.14 | 0.36 |
| 15 | 100.67 | 5.70 | -- | | 8.00 | 0.28 | 8.17 | 0.75 |
| 16 | 99.27 | 5.80 | 101.84 | 5.96 | 8.43 | 0.40 | 8.67 | 0.37 |
| 17 | 110.73 | 7.23 | 113.08 | 6.11 | 9.85 | 0.44 | 9.00 | 0.39 |
| 19 | 108.03 | 6.24 | 103.64 | 6.63 | 10.07 | 0.69 | 9.30 | 0.55 |
| 22 | 103.48 | 5.54 | 91.41 | 5.30 | 9.12 | 0.42 | 8.19 | 0.59 |
| 25 | 106.20 | 11.22 | -- | | 9.00 | 0.80 | 8.00 | 0.58 |
| 28 | 103.45 | 7.43 | 92.63 | 11.95 | 7.67 | 0.93 | 9.50 | 0.79 |
| 29 | 107.56 | 6.61 | 89.48 | 11.86 | 8.21 | 0.38 | 7.55 | 0.45 |
| 32 | -- | | 74.26 | 11.28 | 6.25 | 1.89 | 7.00 | 0.88 |

**Table S6**. Variance components used to estimate $P_{CT}$ (variance between sampling years (VC), variance attributed to populations nested within years (VP) and variation within populations (VI)) for two size (height and number of leaves) and two herbicide resistance traits measured at 1.7 kg a.i./ha glyphosate (surival and biomass). 95% lower and upper confidence limits were estimated by bootstrapping across individuals.

| Trait | $V_C$ | $V_P$ | $V_I$ | $P_{CT}$ | 2.5L | 97.5U |
|---|---|---|---|---|---|---|
| Height | 975.260 | 944.470 | 2682.316 | 0.276 | 0.160 | 0.573 |
| Number of Leaves | 8.174 | 7.367 | 7.881 | 0.403 | 0.000 | 0.760 |
| Survival | 1.400 | 0.227 | 0.170 | 0.810 | 0.063 | 0.932 |
| Biomass | 1.628 | 0.175 | 0.153 | 0.859 | 0.415 | 0.940 |