Received Date : 13-Mar-2015
Revised Date : 28-May-2015
Accepted Date : 16-Jun-2015
Article type : Original Article

The geographic mosaic of herbicide resistance evolution in the common morning glory, *Ipomoea purpurea*: Evidence for resistance hotspots and low genetic differentiation across the landscape

Adam Kuester[1*], Shu-Mei Chang[2], Regina S. Baucom[1]

[1]2059 Kraus Natural Science Building
Department of Ecology and Evolutionary Biology
830 North University
University of Michigan
Ann Arbor, Michigan, 48109
Fax: 1 (734) 763-0544

[2]2502 Plant Sciences Building
Plant Biology Department
University of Georgia
120 Carlton Street
Athens, GA 30602

*Corresponding author

Email Addresses:

AK: kuesteam@umich.edu

SMC: smchang@uga.edu

RSB: rsbaucom@umich.edu

Running head: Geographic mosaic of herbicide resistance

Article type: Original Research article

Abstract

Strong human-mediated selection *via* herbicide application in agroecosystems has repeatedly led to the evolution of resistance in weedy plants. Although resistance can occur among separate populations of a species across the landscape, the spatial scale of resistance in many weeds is often left unexamined. We assessed the potential that resistance to the herbicide glyphosate in the agricultural weed *Ipomoea purpurea* has evolved independently multiple times across its North American range. We examined both adaptive and neutral genetic variation in 44

populations of *I. purpurea* by pairing a replicated dose-response greenhouse experiment with SSR genotyping of experimental individuals. We uncovered a mosaic pattern of resistance across the landscape, with some populations exhibiting high survival post-herbicide and other populations showing high death. SSR genotyping revealed little evidence of isolation by distance and very little neutral genetic structure associated with geography. An approximate Bayesian computation (ABC) analysis uncovered evidence for migration and admixture among populations before the widespread use of glyphosate rather than very recent contemporary gene flow. The pattern of adaptive and neutral genetic variation indicates that resistance in this mixed-mating weed species appears to have evolved in independent hotspots rather than through transmission of resistance alleles across the landscape.

Key Words: Morning Glory, Glyphosate, SSR, Weed, Resistance, *Ipomoea purpurea*, approximate Bayesian computation

Introduction

The evolution of herbicide resistance in weedy plants is an excellent example of adaptation to strong human-mediated selection (Vigueira et al., 2013), and one that, like other examples of resistance to xenobiotics, carries an immense ecological and economic cost. Over 230 cases of resistance have evolved in the relatively short period of time in which herbicides have been utilized for weed control (Heap, 2014). This resistance evolution, in practical terms, can mean a dramatic loss of efficacy of weed control for large areas of land, as well as a concomitant change in the weed populations inhabiting both crop fields and crop margins (Culpepper, 2006; Webster & Nichols, 2012). In addition to providing novel study systems for

rapid evolutionary change, examination of the forces underlying the evolution of herbicide resistance across populations is key to developing strategies for reducing their impact—one that is estimated to be as high as 33 billion USD, yearly (Pimentel et al. 2005).

The ability of a population to adapt to the strong selection from herbicide application is ultimately dependent on the amount and type of genetic variation that is available to selection (Jasieniuk et al., 1996; Delye et al., 2013). Thus the population size, genetic architecture, standing genetic variation, amount of gene flow, and the mutation rate (Hedrick et al., 1976) are all interacting factors that dictate the emergence of resistance across populations. While it has been hypothesized that gene flow between populations may be a more likely cause than novel mutations for the appearance of resistance across the landscape (Jasieniuk et al., 1996), of the few species that have been investigated, only highly self-pollinating species exhibit isolation by distance in the level of resistance—a pattern consistent with the idea that gene flow contributes to the spread of resistance alleles (Osuna et al., 2011; Okada et al., 2013). In comparison, the outcrossing grass species *Alopecurus myosuroides* exhibits a mosaic resistance pattern and no evidence of isolation by distance across populations, suggesting that resistance has evolved independently on a local scale (Delye et al., 2010). Unfortunately, because the majority of herbicide resistance studies are generally either descriptive examinations of the level of resistance across an often limited number of natural populations (Beckie et al., 2000; Preston & Powles, 2002; Neve & Powles 2005; Bernards et al., 2012), or investigations of the molecular basis of resistance (Marshall & Moss, 2008; Cseh et al., 2009; Beckie et al., 2011; Lang et al., 2011; Sada et al., 2013), we currently have a very limited view of how within- and between-population processes, such as gene flow and heterogeneous selection can influence herbicide resistance evolution across the landscape. Such examinations are, to our knowledge, lacking in species that employ mixed mating systems even though ~32% of weedy plants exhibit a mixed-mating strategy (Kuester et al., 2014). Thus, in addition to the need for more examinations of resistance evolution across the landscape, we also have a broad gap in our understanding of the spatial context of resistance evolution in species that are predominately insect pollinated and/or exhibit mixed mating.

The study of the spatial scale of herbicide resistance is relevant to both basic evolutionary biologists and applied scientists for somewhat disparate reasons: evolutionary researchers are fascinated by the repeatability of the evolutionary process whereas applied scientists, who want

to maintain low levels of resistance in nature, need to understand where control efforts are best implemented. For example, different management recommendations would be made if resistance evolved in a single population and spread compared to a scenario where herbicide resistance evolved independently in separate populations. To determine which scenario is most likely for a given weed species, researchers generally pair an assessment of the level of resistance across populations collected from the landscape, often in a common garden study, with an examination of the pattern of neutral genetic variation across these same populations. If, for example, the level of resistance displays a pattern of isolation by distance, one can infer that resistance is spread by gene flow either on a local scale or at greater distances; an assessment of neutral genetic variation that likewise identifies isolation by distance would add further weight to the idea that gene flow is responsible for the spread of resistance. If, as in *A. myosuroides*, a mosaic pattern of resistance is identified such that highly resistant populations are located in close proximity to susceptible populations (*i.e.*, no evidence of isolation by distance and variation in resistance across populations), then it is inferred that populations are independently evolving resistance across the landscape. In this case, a pattern of high neutral genetic structure across populations would suggest rather limited gene exchange among populations, supporting the possibility that populations are independent evolutionary units. Hence, pairing an assessment of the level of resistance across the landscape with investigation of the genetic structure of a weed can allow us to identify the evolutionary units of herbicide resistance (Menchari et al., 2007)—and, likewise, provide initial information regarding the repeatability of the evolutionary process (Ralph & Coop, 2010). Such examinations may also give insight into the ability of populations to respond to other abiotic and biotic selective agents following extreme bouts of selection (e.g., the likelihood of evolutionary rescue; Gonzalez et al., 2013). Finally, once the level of resistance and patterns of neutral genetic differentiation are characterized across the landscape, the more challenging question of how resistance has arisen—*i.e.*, through selection on standing genetic variation, or due to novel mutations across populations—can be addressed.

Ipomoea purpurea, the common morning glory, is a competitive crop weed within the Southeastern and Midwestern regions of the US (Defelice, 2001). This species has become an increasingly problematic species since the increased use of glyphosate (Culpepper, 2006; Webster & Nichols, 2012), which is the main ingredient in the widely used herbicide RoundUp. Previous work has uncovered both additive genetic variation in resistance to glyphosate and

positive selection on resistance showing that the criteria for the evolution of a higher level of resistance are met (Baucom & Mauricio, 2008). Additionally, historically preserved accessions exhibit genetic variability in herbicide defense, suggesting that the ability to evolve resistance in this species was present ancestrally (Baucom & Mauricio, 2010). Although this species is considered an emerging glyphosate resistant weed (reviewed by Sandermann, 2006), we currently do not know if the level resistance varies among populations across the species' range, nor do we know the extent to which populations may be connected *via* gene flow across the landscape. Here, and as part of our broader goal to determine if resistance in this species has arisen from independent, novel mutations within separate populations, different regimes of selection across farms, or has spread *via* gene flow from a single or few sources, we examine both the level of herbicide resistance and the structure of neutral genetic variation across many natural populations of its range across North America. We performed a replicated glyphosate dose-response experiment and assessed the pattern of neutral genetic variation within this species using microsatellite markers to address the following specific questions: (1) Is there a geographic mosaic pattern of glyphosate resistance in *I. purpurea*, indicating that resistance has evolved independently in separate populations across the landscape, or, is there a pattern of isolation by distance suggesting a single origin? (2) Does the pattern of neutral genetic structure across this species' range provide evidence that populations are genetically isolated or that gene flow, whether historical or contemporary, has occurred or is occurring? and (3) Is there evidence for migration between populations of the southeastern US after glyphosate was put into wide-spread use across the landscape, indicating that contemporary gene flow is responsible for the spread of resistance? While the majority of studies that assess neutral genetic variation in herbicide resistant weeds have investigated either predominantly outcrossing, wind-pollinated species, or alternatively highly selfing species, the work presented herein considers a species that exhibits a mixed-mating system, and, one that, by all indications, is in the early stages of glyphosate resistant evolution across the landscape.

Materials and Methods

Field collections and greenhouse resistance screens—We collected leaf material and seeds from 44 populations of *I. purpurea* located within soy, cotton, corn or alfalfa fields selected at random from six states across the Midwestern and Southeastern US (IN, OH, VA, NC, SC, TN; Figure

1a, Table S1). We collected between 20-40 seeds and leaf material from a single maternal plant every 2 meters at each of our 44 sites until we had sampled from at least 30 individuals per population. Each population was sampled from a discrete agricultural field, which we assume to represent discrete units of selection. Populations were at least 5 km apart.

We planted two experiments to assay herbicide resistance across populations on June 11, 2013 in separate greenhouses at the University of Georgia Plant Biology Greenhouses (Athens, GA). Ten seeds from each population (one seed per maternal line) were scarified then planted in pine bark soil in SC10 super conetainers (Stuewe and Sons, Tangent, OR) in each of six experimental treatments, described below. Individual plants were randomly assigned to racks that were then randomly assigned to flow trays (4 racks per flow tray). Pots were watered daily and flow trays were filled with water to prevent desiccation. Only plants that germinated prior to June 26, 2013, were included in the experiment—however, germination was high (88% overall) in both experiments and ranged from 50-100% among populations. A total of 4,614 plants were used in our resistance assays, with 1,995 and 2,619 individuals planted in each experiment. The number of individuals per population and treatment combination used in the experiment can be found in Table S1 and Table S2, respectively.

We measured the height of the stem and number of leaves of each individual three weeks after planting, when the majority of individuals were at the 5-leaf stage. Average height of the plants prior to herbicide application was 13.1±0.3 cm. Plants were then sprayed with RoundUp PowerMax (Monsanto, St Louis, MO) at rates around the recommended field rate (1.54 kg ai/ha) of 0, 0.21, 0.42, 0.84, 1.70 and 3.40 kg a.i./ha (the 0 kg a.i./ha treatment was used as a water control) using a hand-held, CO_2 pressurized sprayer (Spraying Systems Co., Wheaton, IL). The same applicator treated each of the two replicate experiments. We sprayed plants at a rate of 187 liters/ ha at 30 psi with a stride pace of 90 paces per minute at 1.5 meters above the plants. Three weeks after glyphosate application we scored survival and the height of the living stem of each plant.

DNA extraction and genotyping—We isolated DNA from approximately 18 maternal lines per population using a modified CTAB protocol developed by T. Culley (pers. comm.). All DNA samples were quantified by spectrophotometry and diluted to 20 ng/µl for subsequent PCR. We identified 15 SSR loci that showed compatibility for multiplexing and were easily scorable of 20 that were previously described for *I. purpurea* (Table S3; Kuester et al. 2012). Forward primers

were fluorescently tagged with 6-FAM, VIC, NED or PET. All unlabeled and 6-FAM primers were obtained from Integrated DNA Technologies (Corralville, IA). The VIC, NED, and PET primers were purchased from Life Technologies (Carlsbad, CA).

Because fragment size and dye incompatibilities precluded running all 15 loci in one multiplex, we split loci into two multiplex PCR reactions. One multiplex consisted of 0.15 μM of IP8, IP2, IP27, 0.20 μM of IP31, and 0.25 μM of IP34, IP18 and IP1. The second multiplex consisted of 0.05 μM IP36, 0.10 μM of IP47, 0.15 μM of IP6, and 0.25 μM of IP12, IP21, IP45, IP26, and IP42. Ten μL PCR reactions were run with Qiagen Master Mix (Valencia, CA). Thermocycler conditions consisted of 95 ºC for 3 minutes, 35 cycles of 94 ºC for 30 s, 50 ºC for 90 s, 72 ºC for 60 s, and a final extension at 72 ºC for 10 minutes on an Eppendorf (New York, New York) MasterCycler Pro thermacycler. One μl of PCR product was used for fragment detection using an Applied Biosystems 3730 DNA Analyzer (Carlsbad, CA) at the Cornell Life Sciences Core Facility (Ithaca, NY). An ABI GS500 for multiplex 1 and GS600 for multiplex 2 size standards were used for fragment length comparison. All sample genotypes were analyzed using Applied Biosystems PeakScanner 1.0 analytical software (Carlsbad, CA). A PP (Primer Peaks adjustment) sizing default was used for the analysis.

Data Analysis

We included populations with at least 10 germinants per experiment (all treatments combined) in the analyses of spatial variation of herbicide resistance. We first report a species dose response curve and then follow with patterns of survival across populations at doses above the recommended field rate.

Dose response curve—Preliminary analysis showed a significant effect of replicate experiment on the proportion that died. Thus, we used residual values after accounting for the effect of greenhouse experiment to estimate our dose response curves. The use of residual is recommended by Kelly & Rice (1990) to smooth curves in dose response analyses, and has been performed in other dose response contexts (Kilsby et al., 2000). Replicate experiments differed primarily due to a higher rate of death in one greenhouse at both the 1.7 and 3.4 kg a.i./ha herbicide levels, but the correlation between proportion survival per population across each greenhouse was moderate and significantly different from zero across all concentrations (R =

0.480, P = 0.004), showing that although we identify differences in the survival of plants in separate greenhouse experiments, the populations performed similarly relative to one another in the different experiments. To estimate the dose response curve, we first fit the residuals by regressing a general linear model of survivorship on experiment with a binomial distribution to account for the effect of replicate. The residual data were then fit to a Weibull 2.4 parameter model (Weibull, 1951) using the drc package (Ritz & Streibig, 2013) in R (R Development Core Team, 2012). The Weibull 2 model was used to extrapolate the effective dose at eliminating 50% of a population (ED_{50}) was expressed as:

$Y = c + (d-c)(1-\exp(b(\log(x)-\log(e))))$

Where Y is the response (survivorship), c is the upper limit of the curve, d is the lower limit of the curve, e is the ED_{50}, and b is the relative slope around e. We first estimate a species-level ED_{50} using all individuals, followed by a regional (Southeastern and Midwestern US) ED_{50} value, and ED_{50} values per state. We included state in the models because we hypothesized that management policies and herbicide procedures might vary at the state level, and this could influence the level of resistance among states.

Survival following herbicide application—We assessed survival at the 1.7 and 3.4 kg a.i./ha treatment levels (a rate that is similar to the suggested field rate of 1.54 kg a.i./ha and a dose that is twice that) to determine if there was a significant effect of population, state, and/or region of origin on survival post-herbicide. To do this, survival was modeled as a binary character (1/0) in a generalized linear model with a binomial distribution using the lmer option of the lme4 package (Bates, 2010) in R (R Development Core Team, 2012). Fixed effects in the model were herbicide treatment, replicate experiment and state while random variables included population nested within state and the interaction between population and herbicide treatment. We estimated a 95% confidence interval for the species survival by bootstrapping across populations using a non-parametric bootstrapping method in the boot package (Canty & Ripley, 2014) in R (R Development Core Team, 2012).

Spatial autocorrelation—We calculated Moran's I to determine if there was a correlation between survival following herbicide application and geographic distance. Specifically, we calculated the correlation between survivorship and its spatial lag using the Spatial

Autocorrelation Analysis in Macroecology (SAM 4.0, Rangel et al., 2010). The significance of the I value was determined by permuting around 0, where a value of 0 would reflect no spatial pattern (or spatial dispersion) in the data (Bivand et al., 2008). We further performed a principle coordinates analysis (PCoA) on the absolute value of the pairwise difference in survival (ΔResistance = abs($Rpopulation_i - Rpopulation_j$) using the covariance standardized option in GenAlEx (Peakall & Smouse, 2006) across populations to determine if populations clustered according to geographic origin or to resistance status (*i.e.* survival).

SSR error rate, reliability, independence and neutrality—We evaluated SSR loci for reliability, independence, and neutrality under mutation-drift equilibrium to make sure interpretations of downstream analyses were appropriate (*i.e.* assumptions made by analyses were not grossly violated). We used MicroChecker (Van Oosterhout et al., 2004) to check for scoring errors per population, which could result from stuttering, large allele drop outs, and null alleles (DeWoody et al., 2006). Each SSR locus was tested for Hardy-Weinberg Equilibrium (HWE) per population using the Hardy Weinberg Exact Test in Genepop on the Web (Sub-option 3: Probability test; Raymond & Rousset, 1995; Rousset, 2008). SSR loci were also tested for linkage disequilibrium for each pair of loci in each population using the genotypic linkage disequilibrium test with default Markov chain parameters in Genepop. A global test of LD for each pair of loci was performed across populations using Fisher's method. We then applied a sequential Bonferroni correction (Miller, 1981) to correct for multiple tests. Loci were independently scored twice to check for accuracy, and we recorded the scoring error within the species for 200 samples. Loci that did not amplify after two independent attempts were scored as missing data; the frequency of missing data for each locus is reported in Table S3.

Genetic differentiation—We assessed patterns of neutral genetic variation across this species' range using a variety of metrics to determine if genetic diversity and population structure were influenced by recent selection *via* herbicide application and, in addition, to assess the likelihood that gene flow could introduce, or has historically introduced, resistance alleles to once-susceptible populations. We first determined the genetic structure of this species by estimating Weir & Cockerham's (1984) θ using SPAGeDi-1.2 (Hardy & Vekemans, 2002). A confidence interval around the global F-statistic was estimated with 1000 permutation per locus and 1000

jackknifed replicates to detect significant deviation from 0. This confidence interval was used to compare with previous estimates of differentiation within the species (Epperson & Clegg, 1987). We evaluated multi-locus estimates of R_{ST}, a measure of genetic differentiation using a step-wise mutation model of marker evolution. Pending no significant difference between R_{ST} and F_{ST} estimates, we report only differentiation using those based on F_{ST}. In preliminary analyses, we assessed the influence of null alleles on our measure of genetic differentiation using FreeNA (Chapuis & Estoup, 2007) by excluding null alleles and comparing F_{ST} to non-adjusted estimates. Since the two estimates were comparable, we report a non-adjusted F_{ST} (95% C.I. F_{STadj}: 0.071-0.171, 95% C.I. $F_{STunadj}$: 0.071-0.168).

We next implemented a hierarchical AMOVA to test the level of genetic differentiation across regions (MW, SE), states within regions (IN, OH, VA, SC, NC, TN), and populations nested within states using GenAlEx v. 6.1 (Peakall & Smouse, 2006). To assess the potential for contemporary or historical admixture between populations, we clustered individuals across the Southeastern and Midwestern US using STRUCTURE v. 2.2.3 (Pritchard et al., 2000) with an admixture model and an MCMC length of 400,000 iterations (100,000 burn-in). Likelihood values of the number of clusters, Ln(P(D)), were assessed from five runs using a range of k values from 1 through 35. We used the delta-k method (Evanno et al., 2005) to determine the most likely number of clusters in our data set. We also performed a principal coordinates analysis to determine if the neutral genetic variation of populations clustered together according to geographical distance in GenAlEx v. 6.1 (Peakall & Smouse, 2006)), and further, tested for isolation by distance using Rousset's (1997) linearized F_{ST}, $F_{ST}/(1-F_{ST})$, and Cavalli-Sforza distance over the natural log of geographic distance using ISOLDE, Option 6 in GenePop (Raymond & Rousset, 1995; Rousset, 2008).

We then performed pairwise comparisons of genetic structure between populations to determine if populations were significantly differentiated from one another and thus less likely to share a similar evolutionary history. Pair-wise estimates of genetic differentiation among all sampled populations were estimated by F_{ST} (Weir & Cockerham, 1984) using the program FSTAT v. 2.9.3 (Goudet, 2001), and their significance was determined using 1000 permutations and a sequential Bonferroni correction for multiple tests. Finally, we estimated Nei's pairwise genetic distance and a subsequent principle coordinates analysis using the covariance standardized option in GenAlEx v. 6.1 (Peakall & Smouse, 2006).

Approximate Bayesian computation (ABC) analysis—We next used a Bayesian coalescent approach (approximate Bayesian computation; Beaumont, 2010) to examine the likelihood that migration occurred recently between resistant populations in the southeastern US (see *Results*) compared to a scenario of gene flow between populations prior to the widespread use of glyphosate across the landscape. If the former scenario were more likely, we would reason that contemporary gene flow (such as through the movement of contaminating morning glory seed between farms in seed lots) is likely to be responsible for resistance across populations. If, however, the latter scenario of migration before the widespread use of glyphosate were the more likely one, we would infer that resistance has independently evolved in separate populations. Although ABC analysis can be used to make inferences about complex population histories, estimate population parameters such as effective population size (Tallmon et al., 2008), and, has recently been used to model many different scenarios of herbicide resistance evolution (Okada et al., 2013), we elected to model the relatively simple alternative scenarios of migration between *I. purpurea* populations pre- or post-glyphosate use. We employed the software DIYABC v 2 (Cornuet et al., 2008) to test three scenarios (Figure 2) using the microsatellite data from North Carolina and Tennessee populations—two areas of the landscape where we observed the highest resistance (see *Results*). The first scenario assumed no admixture across populations. The second scenario assumed admixture before the use of glyphosate (at time t4) and a third scenario assumed admixture after the use of glyphosate (time t5) within NC and TN regions.

We estimated t5 as the number of generations that have occurred since 1974 (a range of 2-38), which is the year that RoundUp was approved for chemical weed control (Duke & Powles, 2008). We included a bottleneck event across all populations around the start of glyphosate use in 1974, where population size reduced by 90%, a target value of many herbicides (denoted by dashed line at time t3), from an initial effective population (N_e) size ranging between 250-1000 individuals, which encompasses the range of previously estimated N_e for *I. purpurea* (Gonzales et al., 2012). Additional time points at which weedy populations were first observed in the region and diverged (t1 and t2, 180- 210 generations from present) had previously been described in Defelice (2001) and were included in the models. Parameter values for tested models can be found in Table S4. The probability estimates of model scenarios were compared using posterior probabilities from a local logistic regression of the scenario set, and

100,000 runs were assessed per scenario. We ran these simulations using 15 populations over four trials with four populations per trial. Each trial included two randomly chosen populations from TN and NC each with one population (30) used twice across trials.

Results

Dose-response–The overall species-level ED_{50} estimate for *I. purpurea*, based on survival, was 1.6 kg a.i./ha (95% CI: 1.12-2.10), which is similar to the manufacturer's recommended field dose of 1.54 kg a.i./ha. Twelve populations, all of which were from VA, SC, NC and TN exhibited a proportion survival that was significantly higher than the species average, *i.e.*, resistance values that were greater than the species 95% CI (Figure 1; Figure 3a). Nineteen populations fell significantly below the species average—12 of these were from the Southeastern US (SC, NC, and TN) and seven were from the Midwestern US (IN and OH) (Figure 3a). Population-level ED_{50} estimates were considerably variable; however, overall, populations within the Southeastern US, principally North Carolina and Tennessee exhibited ED_{50} values above the species-level 95% CI, whereas populations in the Midwestern US, mainly Ohio and Indiana exhibited response levels below the average (Table S5) though the difference was not statistically significant.

Spatial variation in resistance—Although the species' ED_{50} value was very close to the recommended field dose, we found a significant effect of population of origin ($\chi^2 = 145.34$, $P < 0.001$) and state on survival ($F_{5, 8182} = 2.540$; $P = 0.030$, Table 1), indicating the presence of geographic variation in the level of resistance. We found no effect of region, although we observed that northern sites tended to exhibit lower survival than southern populations at 1.7 and 3.4 kg a.i./ha (Figure 1a and 1b and Figures 3a and 3b, respectively). The interaction between population and herbicide dose (Population × Treatment $\chi^2 = 0.040$; $P = 0.980$, Table 1) was not significant, suggesting that even though some populations exhibited higher survival then others, the populations responded in a relatively consistent manner to the different herbicide doses, namely, increasing death at a higher herbicide application rate. There was significant spatial autocorrelation of resistance at distances at a local scale (within 40 miles, Moran's $I = 0.829$, $P = 0.013$), but we observed no isolation by geographic distance across all sampled populations in survival ($R = 0.020$, $P = 0.269$). The Southeastern US exhibits greater diversity in the proportion

survival per population than the Midwestern US; though the most resistant populations do not, in general, cluster together in a PCoA (Figure 4). An exception to this was the highly resistant TN populations, which tended to cluster together.

Genetic diversity and differentiation—Information on scoring errors, deviations from Hardy-Weinberg Equilibrium and locus pair linkage disequilibrium can be found in the 'Supplemental Materials' section. The number of alleles per locus x population ranged from 1.60 to 2.27 (mean = 2.00), and allelic richness per multilocus genotype x population combination ranged between 1.23 and 1.37 (mean = 1.30). Expected (H_e) and observed (H_o) heterozygosity ranged between 0.230-0.372 (mean = 0.304) and 0.191-0.400 (mean = 0.294), respectively (TableS6).

The AMOVA uncovered evidence for low but significant genetic differentiation across region (F_{RT} = 0.043, P = 0.001, Table 2), states within regions (F_{SR} = 0.119, P = 0.001) and populations within states (F_{PS} = 0.157, P = 0.001). The majority of genetic variation in *I. purpurea* is found within populations (F_{IT} = 0.428, P = 0.001).

We estimated Weir and Cockerham's (1984) F_{ST} across the species' range to be 0.127, (95% CI: 0.071-0.183), which is lower than a previous estimate using floral color (F_{ST} = 0.218, Epperson and Clegg 1987). We detected no difference between R_{ST} and F_{ST} estimates (R_{ST} = 0.068, 95 % CI: 0.0681- 0.122). One hundred and eight (21%) of 595 pairwise-F_{ST} values between populations were significantly greater than 0, and ranged from 0.035 (Burgaw, NC and IN10; Table S7) to 0.274 (Hare Road, NC- Willis Grove, TN; Table S7). We found no evidence of genetic differentiation among 79% of populations; the majority of significant F_{ST} values were between populations sampled from different states (86%). Of the significant F_{ST} values among states, the majority were observed between populations in TN and NC (15%) and SC (15%)—interestingly, these were states in which we observed highest levels of resistance. There was, however, no indication that resistant populations exhibited more or less differentiation compared to other populations, since the majority (64%) of the significant pairwise-F_{ST}'s were among populations that exhibited resistance values within the species' 95% CI, and, less than 2% of the significant pairwise- F_{ST}'s were between resistant and susceptible populations.

Further, although we found a moderate level of genetic differentiation across populations sampled from North America, our STRUCTURE analysis uncovered a pattern of widespread migration and admixture among individuals within populations (Figure 5). The most likely

number of genetic clusters within the sampled range for *I. purpurea* was k = 3 (ln(P(D) = -8265.7). All 3 genotypic clusters were found within individuals sampled from North Carolina suggesting that populations within this state are either the source of introduction for other weedy populations, or, this state has had multiple introductions of different seed lots.

Our PCoA of neutral genetic variation revealed a slight clustering of Midwestern US populations, which had similarly been found in the AMOVA result for regional genetic differentiation (F_{RT} = 0.043, P = 0.001). However, these populations were contained within the range of variation across the Southeastern US populations (Figure 6), and the first two axes of the PCoA explained only 8.9% and 6.3% of the variation. Thus, geography explains only a small portion of the neutral genetic diversity of this species, and the majority of neutral genetic variation across this species' range in the US is present within the southern populations.

Wilcoxon tests on the first 2 axes of the principle coordinates found no difference, across either axis, for the populations when assigned either 'resistant' (< 50% death, N = 11) or 'susceptible' (>50% death, N = 22) in PC1 or PC2 mean scores (Axis 1: W = 157, P = 0.175; Axis 2: W = 106, P = 0.585). Hence, there was no indication that the neutral genetic variation of this species clustered according to resistance status rather than geography, as would be expected if propagules from, for example, resistant TN populations had migrated to the resistant Carolina populations and established and/or admixed. We did not uncover evidence of isolation by distance using linearized F_{ST} over geographic distance (R^2 = 0.012, P = 0.142), nor did we uncover significant isolation by distance measured as the Cavalli-Sforza Edwards chord distance (R^2 = 0.010, P = 0.192). Pairwise estimates of Nei's genetic distance similarly did not correlate with geographical distance (R = -0.065, P = 0.11), reinforcing our finding of either widespread gene flow across populations or colonization following a recent bottleneck.

Approximate Bayesian computation analysis—We found overwhelming support for admixture prior to glyphosate use (Table 3, average posterior probability across four trials = 0.9515, 0.9413-0.9617) rather than the scenario of gene flow and admixture after 1974, or the time that glyphosate was put into widespread use (average posterior probability = 0.0474, 0.0372-0.0575). This scenario was also more likely than the scenario of no admixture (average posterior probability = 0.0012, 0.0004-0.0021).

Discussion

Our comprehensive analysis of herbicide resistance and neutral genetic variation in the weed *Ipomoea purpurea* has uncovered four major findings. First, while we find that the overall species ED_{50} value is similar to the recommended field dose, we observed considerable spatial heterogeneity in resistance with some populations exhibiting ~100% survival at high doses of glyphosate and others exhibiting high susceptibility. Second, we found little indication that the level of resistance exhibits isolation by distance suggesting that resistance across populations of this species results from either novel mutations within each population or is a result of differing rates and exposures to herbicide application across the landscape. Strikingly, we uncovered little evidence for a genetic signal via isolation by distance or strong geographic structuring in our assay of neutral genetic variation—we instead detected a pattern of widespread migration and admixture across this species' range in the US. Finally, our ABC analysis indicated that gene flow between populations most likely occurred prior to the wide-spread use of the herbicide rather than very recently. Overall, these results support the idea that some populations of *I. purpurea* have rapidly developed higher levels of resistance to this herbicide within a short time frame (since the wide-spread use of RoundUp beginning in the early 1990's), and, that it is unlikely increased resistance is due to contemporary gene flow between populations, but rather, results from independent regimes of selection *via* the herbicide. We discuss each of these main points below.

The geographic mosaic of herbicide resistance—We uncovered broad variation in resistance across populations collected from the Southeastern and Midwestern US, with a pattern that indicates herbicide resistance is evolving independently in a mosaic of hotspots. Although we found that the species average level of resistance is comparable to the suggested field dose (1.54 kg a.i./ha), we uncovered populations that exhibited very high or very low survivorship post-herbicide application. Populations that exhibited high survival and thus high resistance did not appear to cluster in one region of the landscape—*i.e*, resistant populations were located near susceptible populations—suggesting that resistance has independently evolved across disparate areas of this species' distribution. This pattern of potentially independent resistance hotspots has been shown in other resistant weed species (Menchari et al., 2007; Delye et al., 2010), and can result from differences in management practices across geography (Delye et al., 2010),

differences in the structure of genetic variation within populations across the landscape (Mopper et al., 2000; Brodie et al., 2002; Bernhardsson et al., 2013; Delye et al., 2013) or a combination of differences in herbicide use patterns and variation in the standing genetic variation of populations. That we detected no evidence for isolation by distance in the level of resistance further strengthens the case that resistance has evolved independently several times across populations of this species. We did, however, uncover evidence for local geographic structuring of resistance (within 40 miles). This finding, in addition to the lack of isolation by distance across all populations suggests that the individual farm is the independent unit of resistance evolution, a conclusion that is similar to that of ACCase resistant blackgrass in France (*Alopecurus myosuroides*) (Delye et al., 2010).

Although we uncovered evidence of a geographic mosaic of resistance, we also found regional differences in survivorship—states in the Southeastern US tended to have higher -ED_{50} estimates and survivorship compared to Midwestern US populations. Because management practices are often regulated at the state level, it is possible that the difference in resistance between regions may result from differences in the recommended dose across areas. For example, TN has the highest recommended application rate in corn (0.75-1.5 lb a.i./ha, Steckel et al., 2014) whereas OH and IN have the lowest recommended application rate (0.56-1.12 lb a.i./ha, Loux et al., 2013); our ED_{50} values for TN and OH and IN align with the upper end of these recommended rates (TN = 1.5 lbs a.i./ha; OH =1.12 lbs a.i./ha; IN = 1.12 lbs a.i./ha) as does survival post-herbicide (Figure 1a).

Patterns of genetic variation and population structure—With some notable exceptions (Okada al. 2013), the neutral genetic variation of many weeds exhibits little structure or spatial patterning (*e.g.*, Bommarco et al., 2010; Delye et al., 2010; Campitelli & Stinchcombe, 2012), potentially due to either their recent expansion across the landscape, few barriers to gene flow, or human-mediated modes of dispersal (*e.g.* dispersal through farm machinery or through contaminated crop seed; Thill & Mallory-Smith, 1997; Owen & Zelaya, 2005). While we find evidence for low to moderate genetic structure across populations (F_{ST} = 0.127, P = 0.001), we find little evidence for a geographic pattern to that structure beyond the slight clustering of Midwestern US populations identified in the PCoA. In particular, we found no isolation by

distance within the species, suggesting a scenario of either widespread gene flow between populations or their relatively recent colonization.

We hypothesize that recent colonization and introduction patterns are responsible for the lack of geographic structure in this species. *I. purpurea* is a very popular ornamental that has been re-introduced to the Southeastern US (Defelice, 2001; Fang et al., 2013) many times following flower color domestication (Glover et al., 1996), and this species does particularly well in warm climates as it is native to central Mexico. Thus, the Southeastern US in particular may have experienced repeated re-introduction and establishment of this species, following which subsequent range expansion or colonization into more northern areas occurred. Perhaps the presence of *some* genetic structure and yet evidence for migration and admixture between populations of this species is due to the re-introduction of a limited but variable pool of germplasm, a scenario similar to that posited for *I. purpurea's* sister species, *I. hederacea* (Campetilli & Stinchcombe, 2014). The Carolinas (specifically NC) have a relatively high density of populations of *I. purpurea* compared to other states—and populations within this range contain all of the genotypes that we detected in our survey, suggesting NC as a possible source for subsequent introductions into other areas of the Southeastern and the Midwestern US.

The low level of genetic structure across populations of this species could also be due to contemporary migration and admixture between populations, and this scenario would strongly suggest that gene flow could be a major driver of resistance evolution in this species. If this were the case, however, we would expect to see patterns of isolation by distance in either our phenotypic resistance data or in patterns of neutral genetic variation—admittedly, a line of reasoning that assumes little chance of long distance propagule or pollen movement *via* human influence. Thus, the overall mosaic pattern of phenotypic variation in this system suggests resistance has emerged through independent evolutionary events whereas the genetic data provide little evidence that the populations are genetically independent. To resolve these two patterns, we used a Bayesian coalescent approach and explicitly considered two scenarios of population connectedness—one in which migration among populations occurred primarily before the commercial approval and widespread use of the herbicide (1974; Fig. 1 of Baucom & Mauricio, 2004) and another that examined the probability associated with very recent, post-widespread glyphosate use. This analysis consistently identified support for the pre-herbicide

migration scenario compared to a scenario of recent, and post-herbicide use migration, pointing to the independent evolution of resistance across populations in a mosaic fashion.

An interesting and remaining question is whether or not the potential independent evolution of resistance is due to selection on pre-existing and hence similar genetic variation, or due to novel mutations in the same or different genomic architecture. Previous work in this species has identified genetic variation for glyphosate defense in accessions of *I. purpurea* collected in the 1980's, prior to the widespread use of RoundUp in the early 1990's (Baucom & Mauricio, 2010), such that the genetic potential for resistance was present ancestrally within this species. This would suggest that independent and increasing regimes of selection on standing genetic variation *via* the use of RoundUp is responsible for resistance uncovered in separate populations. Our data taken in a geographic context also show that it is highly unlikely that there was a single origin of resistance, as the landscape of resistance is heterogeneous even at small areas. It is more plausible that rapid and relatively recent (post-Columbian), but still historical gene flow is responsible for the low genetic differentiation. Populations then went through rapid adaptation of increased resistance across separate areas within the past ~20 years due to the prevalent use of Roundup herbicide. To conclusively rule out the possibility that rare gene flow events may have introduced resistance alleles across disparate areas, however, we will need to determine if the genetic basis of resistance across populations differs, and perform an analysis of the phylogeographic history of resistance alleles.

Acknowledgements

We are grateful to L. Elliott, C. Debban, S. Matthews, G. Lor, and M. Boyd for help with planting, maintaining and measuring plants in the greenhouse. This work was funded by USDA NIFA 2013-07191 to RSB and SMC.

Data Archiving Statement

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.p3v3s

Literature Cited

578 Bates, D. M. 2010. lme4: Mixed-effects modeling with R.URL http://lme4. r-forge. r-project.
579 org/book .
580 Baucom, R. S., and Mauricio R. 2004. Fitness costs and benefits of novel herbicide tolerance in a
581 noxious weed. PNAS 101: 13386-90. Baucom R.S., and Mauricio R. 2008. The evolution
582 of novel herbicide tolerance in a noxious weed: the geographic mosaic of selection.
583 Evolutionary Ecology Research 22: 85-101.
584 Baucom R.S., and Mauricio R. 2010. Defense against the herbicide RoundUp predates its
585 widespread use. Evolutionary Ecology Research 12: 131.
586 Beaumont M.A. 2010. Approximate Bayesian computation in evolution and ecology. Annual
587 Review of Ecology, Evolution, and Systematics 41: 379-406.
588 Beckie H.J., Warwick S.I., Sauder C.A., Lozinski C., and Shirriff S. 2011. Occurrence and
589 molecular characterization of acetolactate synthase (ALS) inhibitor-resistant Kochia
590 (*Kochia scoparia*) in western Canada. Weed Technology 25: 170-175.
591 Beckie H.J., Heap I.M., Smeda R.J., and Hall L.M. 2000. Screening for herbicide resistance in
592 weeds. Weed Technology 14: 428-445.
593 Bernards M.L., Crespo R.J., Kruger G.R., Gaussoin R., and Tranel P.J. 2012. A waterhemp
594 (*Amaranthus tuberculatus*) population resistant to 2,4-D. Weed Science 60: 379-384.
595 Bernhardsson C., Robinson K.M., Abreu I.N., Jansson S., Albrectsen B.R., and Ingvarsson P.
596 2013. Geographic structure in metabolome and herbivore community co-occurs with
597 gnetic structure in plant defence genes. Ecology Letters 16: 791-798.
598 Bivand R.S., Pebesma E.J., and Gomez-Rubio V. 2008. Applied spatial data analysis with R,
599 Springer, New York, NY.
600 Bommarco R., Lann M., Danzer U., Polsson K., and Torstensson P. 2010. Genetic and
601 phenotypic differences between thistle populations in response to habitat and weed
602 management practices. Biological Journal of the Linnean Society 99: 797-807.
603 Brodie E.D., Ridenhour B., and Brodie E. 2002. The evolutionary response of predators to
604 dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between
605 garter snakes and newts. Evolution 56: 2067-2082.
606 Campitelli B., and Stinchcombe J.R. 2012. Natural selection maintains a single locus leaf shape
607 cline in ivyleaf morning glory, *Ipomoea hederacea*. Molecular Ecology 22: 552-564.
608 Campitelli B., and Stinchcombe J.R. 2014. Population dynamics and evolutionary history of the

609 weedy vine Ipomoea hederacea in North America. Genes Genomic Genetics doi:
610 doi:10.1534/g3.114.011700.
611 Canty A., and Ripley B. 2014. boot: bootstrap R (S-plus) functions. R package version 1.3-11.
612 Chapuis M-P., and Estoup A. 2007. Microsatellite null alleles and estimation of population
613 differentiation. Molecular Biology and Evolution 24: 621-631.

614 Cornuet J.M., Santos F., Beaumont M.A., Robert C.P., Marin J.M., Balding D.J., and Estoup A.
615 2008. Inferring population history with DIY ABC: a user-friendly approach to
616 approximate Bayesian computation. Bioinformatics 24: 2713-2719.

617 Cseh A., Cernak I., and Taller J. 2009. Molecular characterization of atrazine resistance in
618 common ragweed (*Ambrosia artemisifolia* L.). Journal of Applied Genetics 50: 321-327.
619 Culpepper A.S. 2006. Glyphosate-induced weed shifts 1. Weed Technology 20: 277-281.
620 Defelice M. 2001. Tall morning glory, *Ipomoea purpurea* (L.) Roth - flower or foe? Weed
621 Technology 15, 601-606.
622 Delye C., Michel S., Berard A., Chauvel J., Brunel D., Guillemin J.P., Dessaint F., and Le Corre
623 V. 2010. Geographical variation in resistance to acetyl-coenzyme A carboxylase A
624 inhibiting herbicides across the range of the arable weed *Alopecurus myosuroides*
625 (blackgrass). New Phytologist 186: 1005-1017.
626 Delye C., Jasieniuk M., and Le Corre V. 2013. Deciphering the evolution of herbicide resistance
627 in weeds. Trends in Genetics 29: 649-658.
628 Dewoody J., Nason J.D., and Hipkins V.D. 2006. Mitigating scoring errors in microsatellite data
629 from wild populations. Molecular Ecology Notes 6: 951-957.

630 Duke S.O., and Powles S.B. 2008. Glyphosate: a once-in-a-century herbicide. Pest management
631 science. 64: 319-325.

632 Epperson B.K., and Clegg M.T. 1986. Spatial-autocorrelation analysis of flower color
633 polymorphisms within substructured populations of morning glory (*Ipomoea purpurea*).
634 Epperson B.K., and Clegg M.T. 1987. Frequency-dependent variation for outcrossing rate among
635 flower-color morphs of Ipomoea purpurea. Evolution: 1302-1311.
636 Evanno G., Regnaut S., and Goudet J. 2005. Detecting the number of clusters of individuals

using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611-2620.

Fang Z., Gonzales A.M., Durbin M.L., Meyer K.K., Miller B.H., Volz K.M., Clegg M.T., and Morrell P.L. 2013. Tracing the geographic origins of weedy Ipomoea purpurea in the Southeastern United States. Journal of Heredity 104: 666-677.

Glover D., Durbin M.L., Huttley G., and Clegg M.T. 1996. Genetic diversity in the common morning glory. Plant Species Biology 11: 41-50.

Gonzales A.M., Fang Z., Durbin M.L., Meyer K.K.T., Clegg M.T., and Morrell P.L. 2012. nucleotide sequence diversity of floral pigment genes in Mexican populations of *Ipomoea purpurea* (morning glory) accord with a neutral model of evolution. Journal of Heredity 103: 863-72.

Gonzalez A., Ronce O., Ferriere R., and Hochberg M. E. 2013. Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. Philosophical Transactions of the Royal Society B: Biological Sciences, 368: 20120404.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9. 3).

Hamrick J.L., and Godt M.J.W. 1996. Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society of London. Seres B, Biological Sciences 351: 1291-1298.

Hardy O.J., and Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2: 618-620.

Heap I.M. 2014. The International Survey of Herbicide Resistant Weeds. Online. Internet. Tuesday, January 8, 2014. Available www.weedscience.com

Hedrick P.W., Ginevan M.E., and Ewing E.P. 1976. Genetic polymorphism in heterogeneous environments. Annual review of Ecology and Systematics 7: 1-32.

Jasieniuk M., Brule-Babel A.L., Morrison I.N. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Science 44: 176-193.

Kelly C., and Rice J. 1990. Monotone smoothing with application to dose-response curves and the assessment of synergism. Biometrics 42: 805-820.

Kilsby D.C., Davies K.W., McClure P.J., Adair C., and Anderson W.A. 2000. Bacterial thermal death kinetics based on probability distributions: the heat destruction of Clostridium

botulinum and Salmonella Bedford. Journal of Food Protection 63: 1197-1203.

Kuester A., Foltz B., Lor G., Chang S-M., Bennetzen J.L., and Baucom R.S. 2012. Development and characterization of simple sequence repeat loci for the common morning glory, *Ipomoea purpurea* (Convolvulaceae). Molecular Ecology Resources online.

Kuester A., Conner J.K., Culley T., and Baucom R.S. 2014. How weeds emerge: a taxonomic, phylogenetic, and trait-based examination using US data. New Phytologist 202:1055-68.

Lang Z.F., Shen J.J., Cai S., Zhang J., He J., and Li S.P. 2011. Expression, characterization, and site-directed mutation of a multiple herbicide-resistant acetohydroxyacid synthase (rAHAS) from *Pseudomonas* sp Lm10. Current Microbiology 63: 145-150.

Loux M., Doohan D., Dobbels A.F., Johnson W.G., and Legleiter T.R. 2013. Weed control guide for Ohio and Indiana, Ohio State Univesity.

Marshall R., and Moss S.R. 2008. Characterisation and molecular basis of ALS inhibitor resistance in the grass weed *Alopecurus myosuroides*. Weed Research 48: 439-447.

Menchari Y., Camilleri C., Michel S., Brunel D., Dessaint F., Le Corre V., and Délye C. 2006. Weed response to herbicides: regional-scale distribution of herbicide resistance alleles in the grass weed *Alopecurus myosuroides*. New Phytologist, 171: 861-874.

Menchari Y., Delye C., and Le Corre V. 2007. Genetic variation and population structure in blackgrass (*Alopecurus myosuroides* Huds.), a successful, herbicide-resistant, annual grass weed of winter cereal fields. Molecular Ecology 16: 3161-3172.

Miller R. 1981. Simultaneous statistical inference, 2 ed. Springer Verlag, New York.

Monsanto 2012.RoundUp PowerMax Herbicide: Complete directions for use. Online. Internet. Tuesday, January 8, 2014. Available: http://www.cdms.net/LDat/ld8CC010.pdf

Mopper S., Stiling P., Landau K., Simberloff D., and Zandt P.V. 2000. Spatiotemporal variation in leafminer population structure and adaptation to individual oak trees. Ecology 81: 1577-1587.

Neve P., and Powles S. 2005. High survival frequencies at low herbicide use rates in populations of *Lolium rigidum* result in rapid evolution of herbicide resistance. Heredity 95: 485-492.

Okada M., Hanson B.D., Hembree K.J., Peng Y., Shrestha A., Stewart C.N., Wright S.D., and Jasieniuk M. 2013. Evolution and spread of glyphosate resistance in *Conyza canadensis* in California. Evolutionary Applications 6: 761-777.

699 Osuna M., Okada M., Ahmad R., Fischer A.J., and Jasieniuk M. 2011. Genetic diversity and
700 spread of thiobencarb resistant early watergrass (*Echinochloa oryzoides*) in California.
701 Weed Science 59: 195-201.
702 Owen M.D., and Zelaya I.A. 2005. Herbicide-resistant crops and weed resistance to herbicides.
703 Pest Management Science 61: 301-311.
704 Peakall R., and Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic
705 software for teaching and research. Molecular Ecology Notes 6: 288-295.
706 Pimentel D., Zuniga R., and Morrison D. 2005. Update on the environmental and economic costs
707 associated with alien-invasive species in the United States. Ecological Economics 52:
708 273-288.
709 Preston C., and Powles S.B. 2002. Evolution of herbicide resistance in weeds: initial frequency
710 of target site-based resistance to acetolactate synthase-inhibiting herbicides in *Lolium*
711 *rigidum*. Heredity 88: 8-13.
712 Pritchard J.K., Stephens M., and Donnelly P. 2000. Inference of population structure using
713 multilocus genotype data. Genetics 155, 945-959.
714 R Development Core Team. 2012. R: A language and environment for statistical computing. R
715 Foundation for Statistical Computing, Vienna, Austria. Available: http://www.R-
716 project.org/.
717 Ralph P., and Coop G. 2010. Parallel adaptation: one or many waves of advance of an
718 advantageous allele? Genetics 186: 647-68.
719 Rangel T., Diniz-Filho J.A.F., and Bini L.M. 2010. SAM: a comprehensive application for
720 spatial analysis in macroecology. Ecography 33: 46-50.
721 Raymond M., and Rousset F. 1995. Genepop (version 1.2): population genetics software for
722 exact tests and ecumenicism. Journal of Heredity 86: 248-249.
723 Ritz C, Streibig JC (2013) Package drc. http://cran.r-project.org/web/packages/drc/drc.pdf.
724 Accessed October 21, 2013
725 Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under
726 isolation by distance. Genetics 145: 1219-28. Rousset F. 2008. Genepop007: a complete
727 re-implementation of the genepop software for Windows and Linux. Molecular Ecology
728 Resources 8: 103-106.
729 Sada Y., Ikeda H., Yamato S., and Kizawa S. 2013. Characterization of sulfonylurea-resistant

Schoenoplectus juncoides having a target-site Asp(376) Glu mutation in the acetolactate synthase. Pesticide Biochemistry and Physiology 107: 106-111.

Sandermann H. 2006. Plant biotechnology: ecological case studies on herbicide resistance. Trends in Plant Science 11: 324-328.

Steckel L., Rohdes G.N., Sims B.D., Hayes R.M., McClure A., Mueller T.C., Brown B., Wiggins M., and Senseman S. 2014. Weed control manual for Tennessee. University of Tennessee, Knoxville, TN.

Tallmon D.A., Koyuk A., Luikart G., and Beaumont M.A. 2008. Computer programs: Onesamp: a program to estimate effective population size using approximate Bayesian computation. Molecular Ecology Resources 8: 299-301.

Thill D.C., and Mallory-Smith C.A. 1997. The nature and consequence of weed spread in cropping systems. Weed Science 45, 337-342.

Van Oosterhout C., Hutchinson W.F., Wills D.P., and Shipley P. 2004. Microchecker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4, 535-538.

Vigueira C., Olsen K., and Caicedo A.L. 2013. The red queen in the corn: agricultural weeds as models of rapid adaptive evolution. Heredity 110: 303-311.

Webster T.M., and Nichols R.L. 2012. Changes in the prevalence of weed species in the major agronomic crops of the southern United States: 1994/1995 to 2008/2009. Weed Science 60: 145-157.

Weibull W. 1951. A statistical distribution function of wide applicability. Journal of Applied Mechanics 18: 293-297.

Weir B.S., and Cockerham C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Data Accessibility:

Sampling locations, morphological data and microsatellite data: uploaded to Dryad doi (upon acceptance).

Table 1. Generalized linear mixed effects model of plant survival as a response of the fixed effects of treatment, experimental replicate, state, and random effects of populations (nested

760 within state) and the population x treatment interaction. Shown are the degrees of freedom (df), F
761 or χ^2 statistic and associated P value.

762

Effect	df	F	P
Fixed Effects			
Treatment	5	155.47	<0.001
Replicate	1	8.28	0.004
State	5	2.54	0.026
Random Effects		χ^2	P
Population(State)	2	145.34	<0.001
Population(State) × Treatment	2	0.04	0.980

763

764 Populations used for this test are listed under the column "2012 Survivorship" in Table S1

765 **Table 2.** Analysis of Molecular Variance (AMOVA) of neutral genetic data. Shown are the main
766 effects of Region (Midwestern and Southeastern US), State, Population and Individual, F-
767 statistic, and F and P values.

768

Effect	F-statistic	F	P
Region	F_{RT}	0.043	0.001
State (Region)	F_{SR}	0.119	0.001
Population(State)	F_{PS}	0.157	0.001
Individual	F_{IT}	0.428	0.001

769

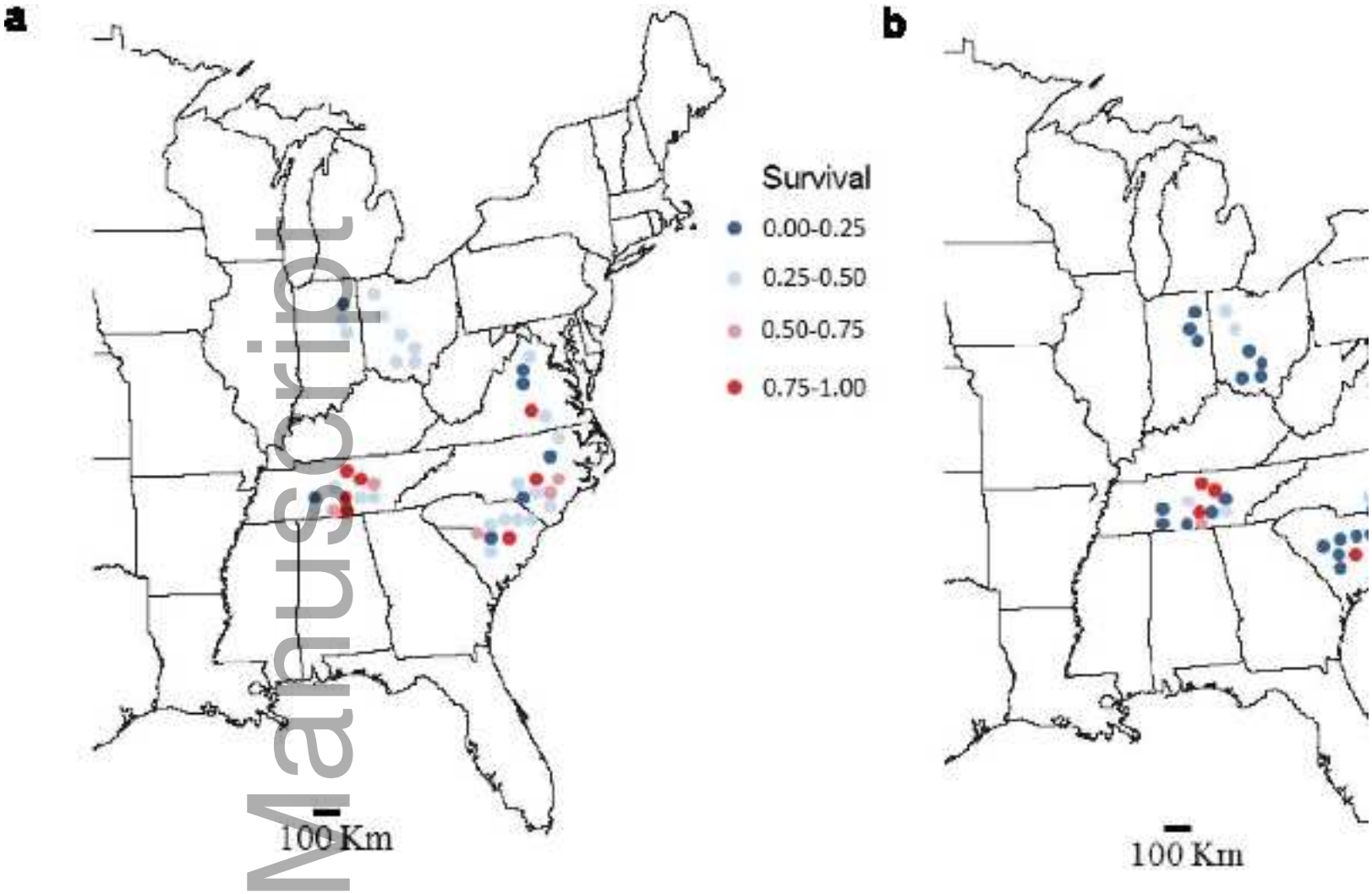
Table 3. The posterior probabilities and associated confidence intervals for different histories of *I. purpurea* populations, based on the logistic estimate from the ABC analysis. Logistic regressions were performed using three scenarios: Scenario 1, no admixture; Scenario 2, admixture before the wide-spread use of the herbicide; and Scenario 3, admixture after the herbicide was put into widespread use in agriculture. The populations used in each trial are shown, along with the posterior probability and associated 95% confidence interval of each scenario for 4 replicate trials and their overall average. Posterior probabilities that are significant are indicated in bold text.

Trial	Populations	Scenario 1		Scenario 2		Scenario 3	
		Posterior Probability	95% Confidence Interval	Posterior Probability	95% Confidence Interval	Posterior Probability	95% Confidence Interval
1	2,14,30,31	0.0001	0.0000-0.0002	**0.9918**	0.9889-0.9948	0.0081	0.0051-0.0110
2	29,4,20,26	0.0005	0.0001-0.0009	**0.9682**	0.9596-0.9768	0.0313	0.0227-0.0398
3	11,10,46,23	0.0001	0.0000-0.0011	**0.9399**	0.9244-0.9554	0.0600	0.0445-0.0755
4	19,21,30,32	0.0039	0.0015-0.0063	**0.9060**	0.8923-0.9196	0.0901	0.0766-0.1036
Average		0.0012	0.0004-0.0021	**0.9515**	0.9413-0.9617	0.0474	0.0372-0.0575

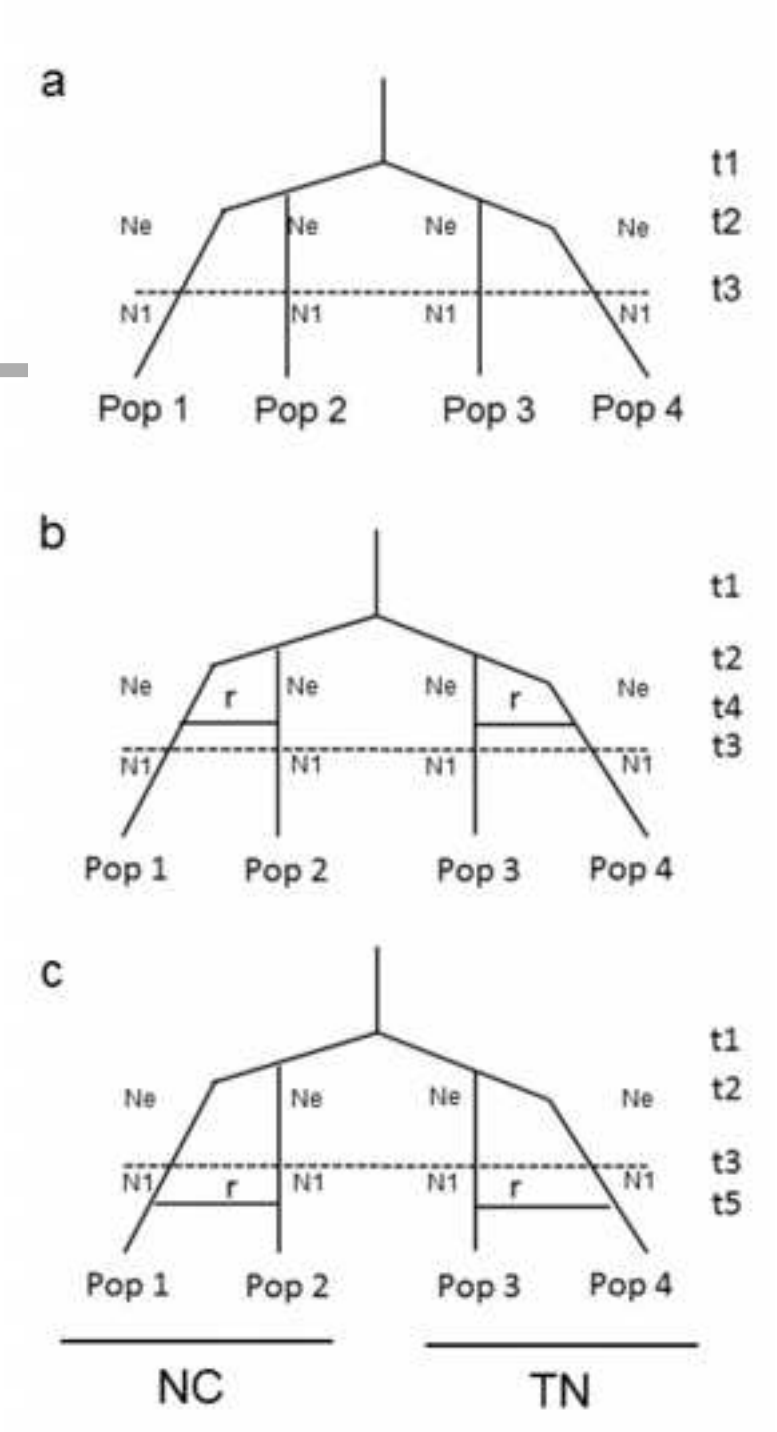
Figure 1. Survival three weeks post- RoundUp herbicide application among *I. purpurea* populations at the rate of (a) 1.7 kg a.i./ha and (b) 3.4 kg a.i/ha. The proportion survival within each population is indicated by color.

Figure 2. Three simple scenarios used in the ABC analyses of *Ipomoea purpurea* populations sampled from NC and TN. Four populations each trial were used to model (a) no gene flow among populations, (b) admixture (denoted r) that occurred before the widespread use of the herbicide, and (c) admixture after widespread glyphosate use. The origin of the populations occurred at t1, or

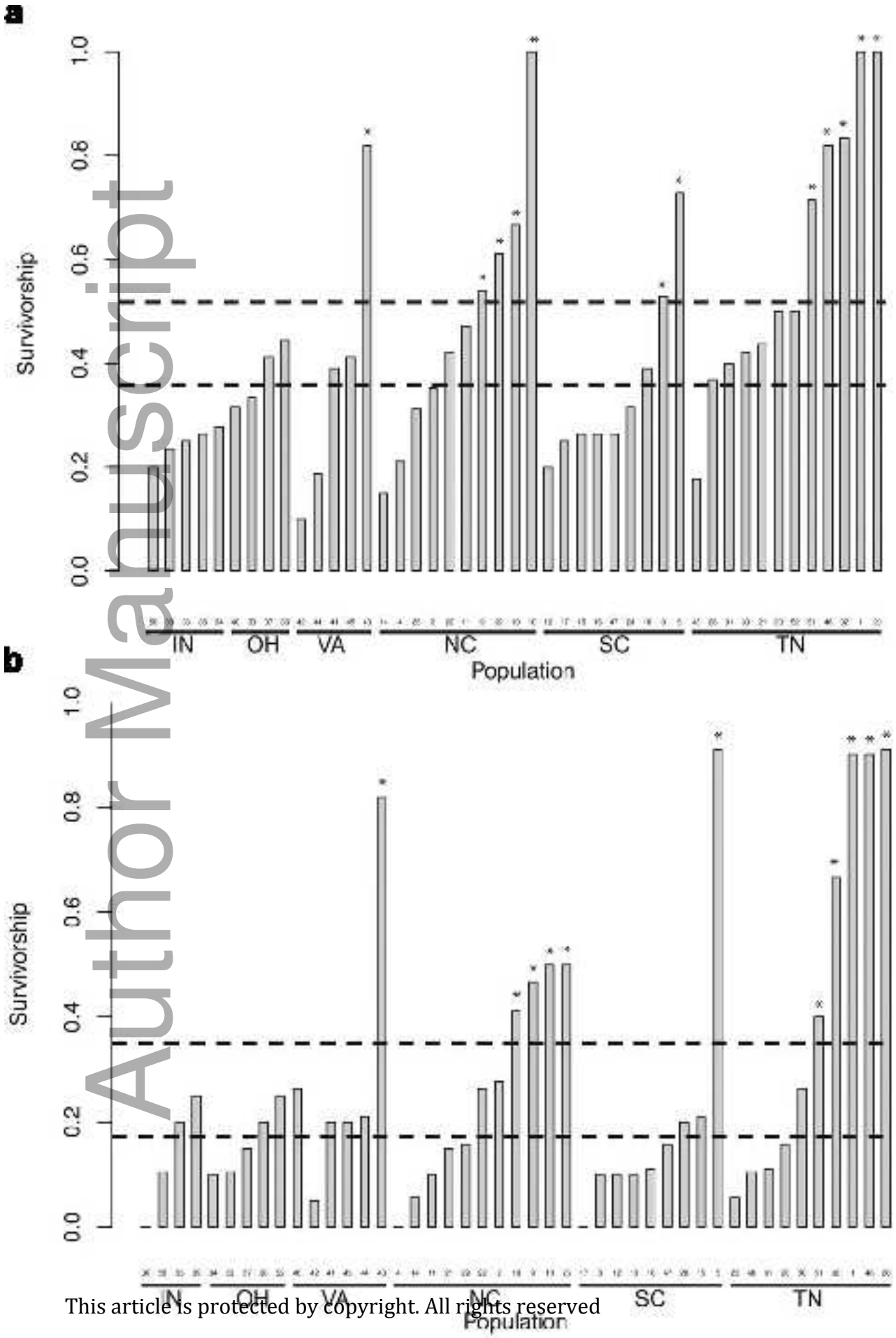
784 the time at which the species was identified as an agricultural weed in the United States, taken from (Defelice 2001). Population
785 divergence between TN and NC populations are indicated by t2. Widespread glyphosate use is indicated in t3 and denoted with a
786 dotted line, corresponding to the year at which glyphosate was released for commercial use in US agriculture (1974). Admixture
787 events are indicated either with t4 (gene flow prior to glyphosate use) or t5 (gene flow after glyphosate use). Also included in the
788 model were the effective population size of each population at t2 (Ne) and subsequent effective size post-glyphosate bottleneck (N1).
789 Parameter estimates are shown in Table S4.
790
791 **Figure 3.** *I. purpurea* populations greater or less than the species' average of resistance at (a) 1.7 and (b) 3.4 kg a.i./ha. Horizontal
792 dashed bars indicate the bootstrap estimates of the 95% confidence interval around the species' mean. Asterisks indicate populations
793 that fall outside the 95% confidence interval.
794
795 **Figure 4.** PCoA of pairwise differences in resistance values between populations at 1.7 kg a.i./ha. Populations are assigned to state
796 (shape), and resistance level by color (red-blue gradient).Coordinate 1 explained 16.4 % and Coordinate 2 explained 13.5 % of the
797 variation in survival. The dashed open circle represents the coordinate space representing all of the Midwestern US populations.
798
799 **Figure 5.** STRUCTURE assignment of individuals to genetic clusters. Small bars represent the assignment of individuals to clusters,
800 with sampling locations differentiated by thick black lines for 35 populations sampled. Shown are each population denoted by State:
801 IN = Indiana, OH = Ohio, NC = North Carolina, SC = South Carolina, TN = Tennessee, VA = Virginia and population ID number.
802
803 **Figure 6.** Principle coordinate analysis of pairwise Nei's genetic distance. Populations are assigned to state (shape) and resistance
804 level by color (red-blue gradient). The proportion of genetic variance explained by coordinates 1 and 2 were 8.9 and 6.3 %,
805 respectively. The dashed open circle represents the coordinate space representing all of the Midwestern US populations.



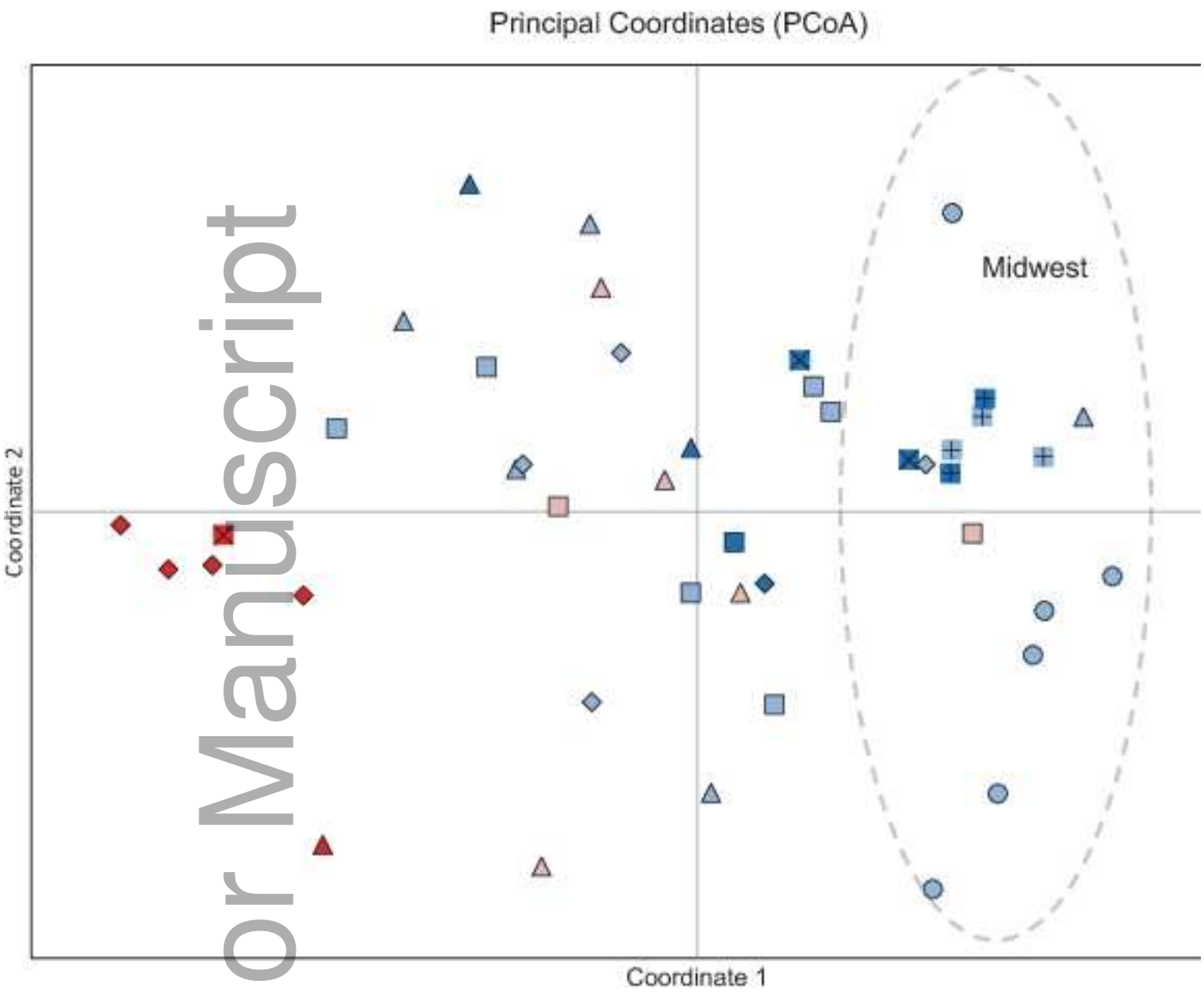
eva12290-fig-0001.eps



eva12290-fig-0002.eps

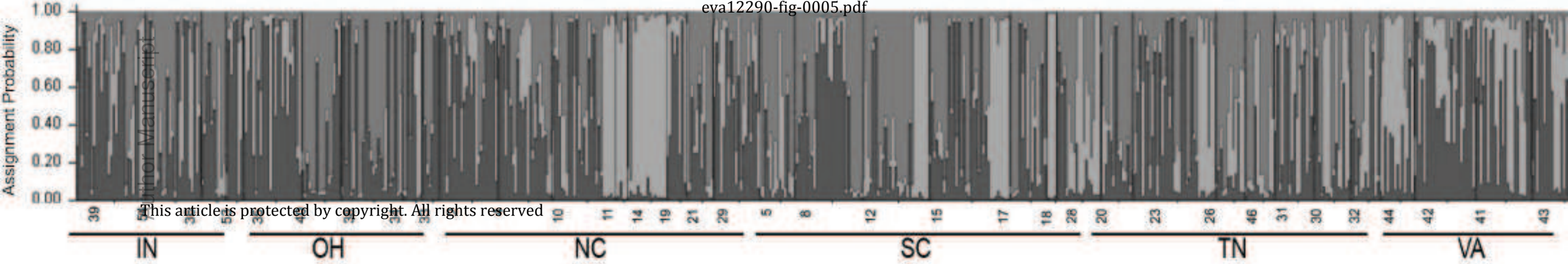


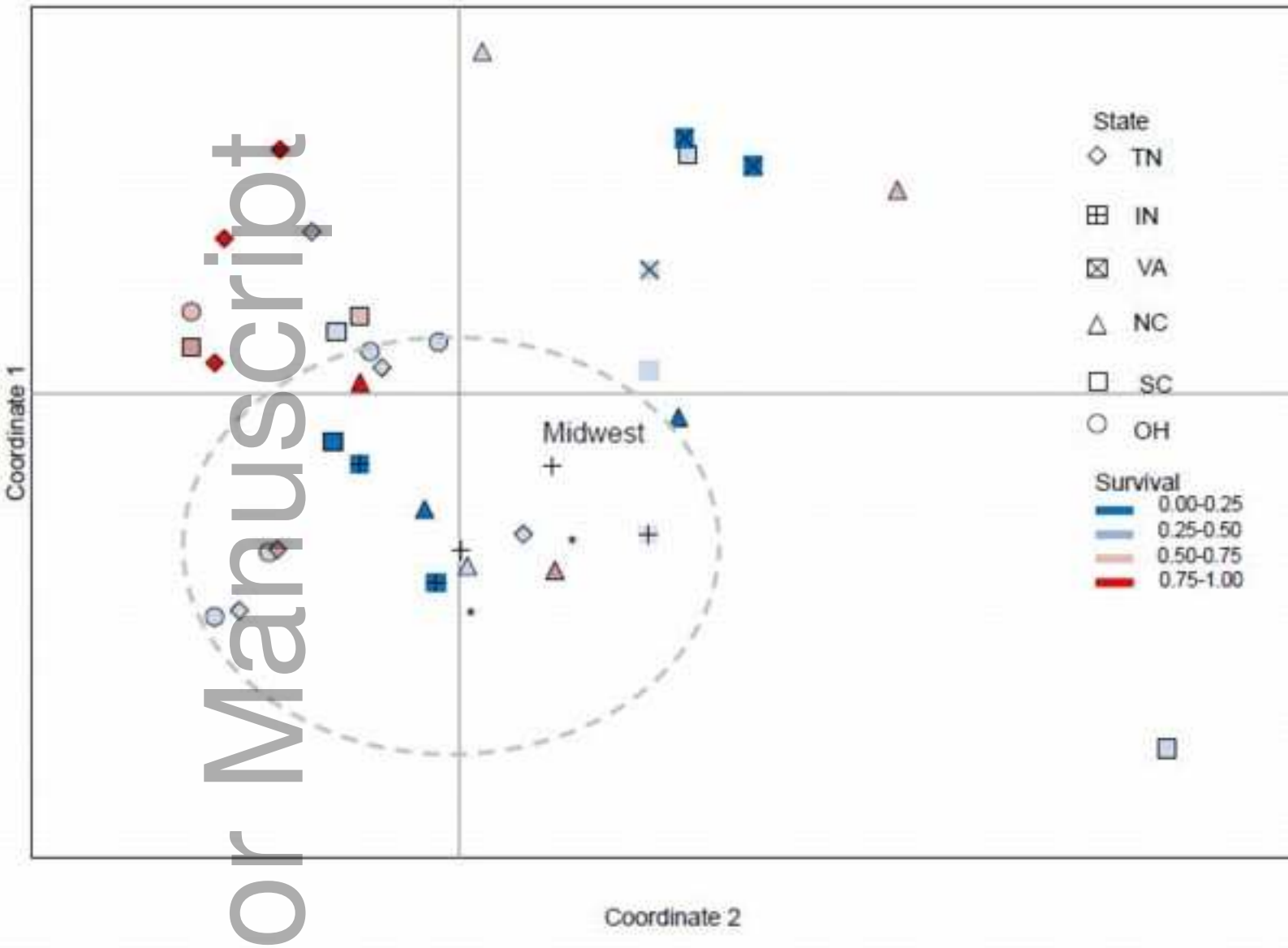
eva12290-fig-0003.eps



eva12290-fig-0004.eps

eva12290-fig-0005.pdf





eva12290-fig-0006.eps