

# The evolution of novel herbicide tolerance in a noxious weed: the geographic mosaic of selection

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**Abstract** Understanding how genetic variation is organized over geography has long been of interest to evolutionary biologists given that traits can vary within and among populations, across regions, and at continental or global scales. The pattern of regional variation can have an important impact on trait evolution at the local or population level. Using a common garden, we asked whether a geographically variable mosaic of tolerance to the widely applied herbicide RoundUp<sup>®</sup> existed in two closely related co-occurring species of morning glory, *Ipomoea purpurea* and *I. hederacea*. We assayed RoundUp tolerance in over 1,700 plants representing 290 families from 29 populations in the southeastern United States. Our findings suggest that the two species of morning glory partition their respective levels of genetic variation for tolerance to glyphosate differently. Variation for tolerance in *I. purpurea* appears to exist among maternal lines and regions, whereas in *I. hederacea*, variation in tolerance existed only among populations. In addition, we find a significant hotspot of tolerance or positive spatial aggregation of this trait on a local scale in *I. purpurea* populations from the Coastal Plain. This suggests that either similar regimes of selection or gene flow between populations can produce a geographic mosaic of tolerance. These results highlight the fact that the genetic variation underlying an adaptive trait can exist at many different scales, whether it be within- or among-populations, among geographical ‘hotspots,’ or among distinct ecological regions. Given these results, the partitioning of genetic variation should be considered before making predictions about an adaptive trait’s evolutionary trajectory.

**Keywords** Tolerance · Genetic variation · Geographic mosaic · Glyphosate · *Ipomoea*

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## Introduction

Ecologists and evolutionary biologists have long recognized the need to incorporate the concepts of spatial scale and geographic structuring as important factors influencing the demographic and genetic patterns of adaptive traits in natural populations (Wright 1943; Hutchinson 1959). This is largely attributable to the recognition that populations are rarely genetically isolated from one another, calling into question the scale at which adaptive traits should be considered.

Variation in adaptive traits can exist among individuals within a single population or among populations physically separated across geography. Variation can also exist among aggregations or groups of populations found over space, giving the appearance of spatial ‘clumping,’ or a mosaic of trait values over a wide geographical area. The resulting pattern of evolutionary ‘hotspots’ or ‘coldspots,’ where neighboring populations exhibit similar high or low trait values, respectively, could be produced by similar regimes of selection within aggregations of populations (Thompson 1997; Brodie Jr et al. 2002). Alternatively, this pattern of spatial variation might be seen if populations are connected by gene flow where the level of variation at one site might potentially be a determinant of evolutionary potential at another site. Larger-scale regional trends can also exist such that populations from ecologically distinct, widely separated areas exhibit differences in trait values.

Despite the recognition that adaptive trait variation can exist at different hierarchical levels, it is most often within a single population that the evolutionary dynamics of an adaptive trait are studied. Considering adaptive trait variation in a broad geographical context will allow greater insight into the evolutionary process; incorporating trait variation data from within and among populations, population aggregations, and ecologically distinct regions will aid further predictions about the evolutionary trajectory of important plant traits.

In a previous study, genetic variation in tolerance to glyphosate, the main ingredient in the herbicide RoundUp<sup>®</sup>, was found within a population of the common morning glory, *Ipomoea purpurea*, a noxious agricultural weed in the southeastern United States (Baucom and Mauricio 2004). Glyphosate use is common in current farming management (Dill 2005) and intense selection by this herbicide could impact the scale at which variation in tolerance resides. For example, if farmers consistently applied the herbicide over many years, we may expect little within-population variation in tolerance, given that populations might be fixed for the highest level of tolerance possible. Under the scenario of intense, continuous selection, populations may exhibit differences in tolerance if they started with different amounts of genetic variation. On a larger geographical scale, if a farmer used the same application rate of glyphosate over multiple areas, aggregations of the level of tolerance might form such that there are hotspots or coldspots across geographical clines. Further, if glyphosate use differs over widely separated geographical areas or geographical regions, differences in glyphosate use or in extant genetic variation for tolerance could potentially promote variation among the regions for level of tolerance. In addition, different regions could potentially serve as reservoirs of tolerance or susceptibility: both scenarios have important evolutionary and management implications.

An initial assessment of where variation in tolerance to glyphosate exists, whether within or among populations, among aggregations of populations leading to a

‘mosaic’ of tolerance, or among ecologically distinct regions will first provide an idea of the potential for the evolution of tolerance in nature. This information will also inform the weed manager of how to best control the continued evolution of the trait. Further, assessing both the level of tolerance and where variation for tolerance resides in more than one species will provide clues as to whether different species can be expected to respond similarly to herbicide application. For example, it would be instructive to examine the level of tolerance as well as the pattern of variation for tolerance in closely related species that co-occur. Since they have experienced similar regimes of selection from herbicide application, differences in both the level of tolerance and the partitioning of variation underlying tolerance might be ascribed to underlying differences between the species, such as their respective mating systems, or patterns of gene flow.

In this paper, we investigate variation in tolerance within and among populations of two morning glory species that have experienced strong selection by glyphosate over the past 25 years. We address the following questions: Over what spatial scale does variation in tolerance to glyphosate exist among populations of two morning glory species: within populations, among populations, or among topographically distinct regions? Is there a geographical mosaic of tolerance in that local populations of each species are found to spatially aggregate for their level of tolerance, such that there are ‘hotspots’ and ‘coldspots’ of tolerance across a wide geographical area? Do closely related species exhibit similar levels of tolerance when found to co-occur, or are the two species exhibiting dissimilar levels of tolerance?

## Materials and methods

### Natural history

The common morning glory, *I. purpurea* (L.) Roth. (Convolvulaceae), is a weedy annual vine that grows in disturbed habitats throughout the southeastern United States. Germination occurs from mid-May to late August. Flowering typically occurs about 6 weeks after germination and continues until the first hard frost. Individual flowers open for a single morning and are pollinated almost exclusively by bumblebees (Ennos 1981), although this species is also capable of self-fertilization. Fruits mature 4–6 weeks after pollination and produce from one to six seeds each. The average outcrossing rate for this species has been estimated as ~70% in natural populations (Ennos 1981; Brown and Clegg 1984; Chang and Rausher 1998).

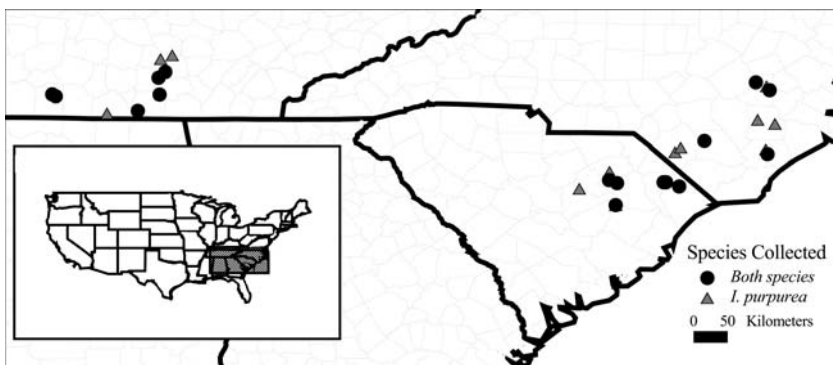
The ivy-leaf morning glory, *I. hederacea* (L.) Jacquin, is also a weedy annual vine which both morphological and molecular data suggests is closely related to *I. purpurea* (Miller et al. 2002, 2004). In the southeastern US, populations of *I. hederacea* are found in disturbed areas and agricultural fields, often alongside populations of *I. purpurea*. Patterns of seed germination and flower and fruit production are similar to those of *I. purpurea*, although *I. hederacea* appears to flower and senesce earlier in the season than *I. purpurea* in natural populations (personal observation). Flowers of *I. hederacea* range from light to dark blue in color and are often visited by bumblebees (Ennos 1981) although the selfing rate for one population has been estimated at 93% (Ennos 1981). The two species do not form viable hybrid offspring from crosses in the greenhouse (Guries 1978) and there is no evidence that the two species successfully mate in the wild.

A review of weed species in the Southeast places annual morning glory species as the most troublesome weeds in soybean and the number two most troublesome weeds in cotton and corn (Webster 2004). Both *I. purpurea* and *I. hederacea* are considered noxious crop weeds that decrease crop yield and lead to harvesting difficulties. Both species are able to tolerate glyphosate in that they are able to survive application of the herbicide, re-grow and produce progeny for the next generation. This tolerance response is a trait distinct from the ability to resist glyphosate application in the same way as the distinction is made in the plant–herbivore literature (Rausher 1992b; Fineblum and Rausher 1995; Mauricio et al. 1997) where resistance prevents damage whereas tolerance ameliorates the effects of damage. By this definition, for example, a plant would be resistant if it had an enzyme that detoxified the herbicide before it could damage the plant. Previously, we examined a single population of *I. purpurea* and found genetic variation for tolerance to glyphosate (Baucom and Mauricio 2004). After the application of the herbicide, *I. purpurea* individuals appeared stunted and damaged, but continue to re-grow and produce flowers.

Glyphosate is a non-specific post-emergence herbicide (Grossbard and Atkinson 1985). It enters the plant through the stems and leaves by diffusion and is mobile throughout the plant in the phloem (Caseley and Coupland 1985). Glyphosate accumulates in the apical meristems and other sites of sugar utilization (Franz et al. 1997), and causes plant death by inhibiting the biosynthesis of aromatic amino acids (Amrhein et al. 1980; Steinrucken and Amrhein 1980) by inhibiting 5-enol-pyruvyl shikimate-3 phosphate synthase, a key enzyme in the shikimate pathway. Approximately 1.5 weeks after application, leaves of sprayed plants begin to exhibit yellowing and necrosis. On many plants, the leaves and the apical meristem completely die, leaving the plant stunted or dead. After being sprayed, plants that survive and produce flowers appear to do so from new stem growth (personal observation).

### Experimental design

Twenty-nine populations of *I. purpurea* and 17 populations of *I. hederacea* were collected from cotton, soybean or corn fields located in either the Coastal Plain in North and South Carolina or from the Cumberland Plateau in middle Tennessee (Fig. 1). The two geographical regions are quite distinct. The average elevation of



**Fig. 1** Locations of populations collected from the Southeast

Coastal Plain populations is about 50 m above sea level, whereas populations from the Cumberland Plateau were found at an elevation greater than 300 m above sea level. The regions also vary in the length of their growing season (Coastal Plain 185–200 days; Cumberland Plateau 175 days), and their average annual temperature (Coastal Plain 55–57 F; Cumberland Plateau 55 F), but not their average precipitation (1,170 mm). The two species were found at a common set of 17 collection sites. Ripened seeds were sampled from up to 20 maternal individuals per species and site.

All populations had a recent history of glyphosate exposure, whether glyphosate was sprayed the same year of seed collection or in the previous year. Farmers and county extension agents were asked about the rate and frequency of glyphosate application within fields. In general, there were no reported differences in the rate of glyphosate application, which was the recommended amount of 1.121 kg a.i. ha<sup>-1</sup>. Variation did exist among the types of crops from which seeds were collected: soy fields were sprayed twice per year, cotton fields were sprayed once during the growing season, and corn was sprayed before the beginning of the growing season. Although variation among crop types could produce variation among populations in level of tolerance, all farmers and extension agents reported the use of crop rotation programs, such that every crop was rotated at least every 2 years. Given the diversity of particular crop rotation regimes, and given that no farmer reported spraying more than 1.121 kg a.i. ha<sup>-1</sup>, we chose to analyze all collection sites together under the assumption that all sites experienced variable selection regimes. Although we attempted to collect from sites that had never experienced herbicide application, we were unable to do so, even along roadsides, which were regularly sprayed with herbicides. The restriction of sampling from only agricultural sites was out of the necessity to reduce the error that would have been introduced by including populations that had experienced herbicide application, yet were exposed to other types of selection regimes, e.g., roadside mowing.

On January 23, 2004, 1,740 field-collected seeds [290 maternal plants, 196 from *I. purpurea* and 94 from *I. hederacea*, 3 replicates per line, 2 treatments (herbicide and no herbicide); see Table 1 for a listing of the number of maternal lines used per species and population] were scarified with a razor blade and planted in a pine-bark soil mixture in a completely randomized design in the University of Georgia Plant Biology greenhouses. Plants were watered daily and kept on a 12:12 day/night light regime with sodium vapor supplementary lights (Energy Technics, York, PA, USA). Plants were fertilized once with a 10-30-20 fertilizer (Peter's Blossom Booster) but otherwise allowed to grow until treatments were applied. On March 1, 2004 glyphosate was applied to individuals at 0 and 1.6 kg active ingredient per hectare with a pressurized CO<sub>2</sub> plot sprayer which keeps droplet size and spray intensity constant (R & D Sprayers, Opelousas, LA, USA).

On March 17 2004, we recorded mortality of the sprayed individuals. We also recorded the date of first flower and the number of flowers produced by each individual daily after glyphosate application in the four treatments. Flowering data was collected over a period of 4 months, which roughly is the length of time the species flower in field conditions. No individuals flowered prior to glyphosate application. Individuals that died in response to the herbicide application were given a fitness score of '0', and individuals that did not germinate prior to application of the herbicide were removed from the analyses. In all analyses, total number of flowers was used as our estimate of individual fitness. Although in a previous study we used the

**Table 1** The population name, state and position of sampled sites, and the number of maternal lines of each species used in the greenhouse experiment

Population name	State	Latitude	Longitude	Number of <i>I. purpurea</i> maternal lines	Number of <i>I. hederacea</i> maternal lines
1. Bergaw1	NC	34.59300	-77.92750	7	0
2. Bergaw2	NC	34.52892	-77.90628	6	2
3. Billings	TN	35.78189	-85.92183	7	0
4. Chicken Rd.	NC	34.55636	-79.12581	8	0
5. Clarendon1	SC	33.85919	-79.91014	7	0
6. Clarendon2	SC	33.85825	-79.91203	6	4
7. Darlington1	SC	34.29872	-79.99122	8	0
8. Darlington2	SC	34.18703	-80.00058	3	6
9. Duplin East	NC	34.92836	-77.80619	8	0
10. Duplin West	NC	34.98214	-78.03550	8	0
11. Florence	SC	34.14800	-79.89383	8	7
12. Good Hope	TN	35.28969	-87.30875	8	8
13. Hare Rd.	NC	35.42436	-77.91706	8	0
14. Horry1	SC	34.10394	-79.07347	6	3
15. Marion1	SC	34.15839	-79.27936	2	8
16. Marion2	SC	34.15878	-79.24639	8	3
17. New Bildad	TN	35.83214	-85.76836	3	0
18. New Hope	NC	35.36983	-77.87783	8	8
19. Old Kenley	NC	35.47292	-78.05972	8	4
20. Red Barn	TN	35.31581	-87.35539	7	8
21. Snakes	TN	35.06758	-86.62953	8	0
22. Spears Soy	TN	35.53350	-85.95214	5	8
23. Starlight	NC	34.61636	-79.05167	8	0
24. Sumter1	SC	34.07639	-80.39094	8	2
25. Sumter2	SC	34.07639	-80.39094	8	0
26. Tar Heel	NC	34.70256	-78.74008	6	6
27. Vervilla	TN	35.60994	-85.86078	8	6
28. Walnut Grove	TN	35.09842	-86.22522	3	3
29. Willis Corn	TN	35.31267	-85.93622	8	8

total number of seeds as the estimate of fitness (Baucom and Mauricio 2004), number of flowers is a more appropriate predictor of reproductive effort in the greenhouse, given that *I. hederacea* is almost a complete selfer whereas *I. purpurea* is primarily outcrossing. For analysis, total number of flowers was relativized by dividing by the average flower number of each species.

Differences in the timing of flowering and seed ripening can be important for a weed in terms of reproducing before crop harvesting. For example, those individuals that are more tolerant to glyphosate will likely recover more quickly from the glyphosate spray and flower earlier than their less-tolerant counterparts. Thus, phenological differences among individuals in response to glyphosate spray are an important component of the variability of response to herbicide application. This variability is included in our estimate of tolerance, in that those that flowered earlier had a higher level of fitness than those that flowered later in the highest level of glyphosate (data not shown).

#### Variation for tolerance

Three mixed model analyses of variance were performed to assess the significance of tolerance at all levels in the geographical hierarchy; one model included both species

and the other analyses of variance were performed per species. The MIXED procedure of the SAS statistical software package (Version 8.0) was used in the analyses with square root  $(1 + y)$  transformed relative fitness as the dependent variable. In the first model, all predictor variables introduced by our method of sampling were included (species, region, population nested within region and species, and maternal line nested within population, region and species) as was the treatment effect. Treatment, species, and region were considered fixed effects whereas population, maternal line, and the interactions between treatment and population or treatment and maternal line were considered random effects. In the first model, we interpret a significant species by treatment interaction as evidence for a species differing in tolerance to glyphosate. A significant species by region by treatment interaction would provide evidence for regional variation among the species in response to glyphosate, although the biological interpretation of three-way interactions can be difficult.

Due to the nested nature of our sampling scheme (maternal lines within population within species) we performed the same mixed model analyses of variance for each species in order to assess whether tolerance varied between the two regions, or among populations and maternal lines. Specifically, we determined if there was a significant interaction between fitness and treatment environment among maternal lines, populations, and regions with maternal line nested within population and region, population nested within region, region, and the interactions between these variables and treatment as the independent variables. Maternal line, population and their interactions with treatment were considered random effects. Region, treatment, and their interactions with treatment were considered fixed effects. Evidence for significant variation in tolerance between the Coastal Plain and Cumberland Plateau regions, and the populations collected within these regions, respectively, were determined by the interaction term between region and treatment, and population and treatment. The interaction term between maternal line and treatment determines if there is genetic variation underlying tolerance (Simms and Triplett 1994; Mauricio et al. 1997).

### Measurement of tolerance

Tolerance was estimated using individuals collected from 29 populations of *I. purpurea* and 17 populations of *I. hederacea*. It is not possible to obtain values of tolerance on an individual plant because any one individual cannot be both damaged and undamaged (Rausher 1992a; Mauricio et al. 1997; Tiffin and Rausher 1999; Stinchcombe and Rausher 2002). Thus, we first estimated tolerance for each maternal line and then averaged these values per population.

Tolerance is most often modeled as a norm of reaction by regressing fitness on damage (see Simms and Triplett 1994; Mauricio et al. 1997; Tiffin and Inouye 2000; Weinig et al. 2003; Baucom and Mauricio 2004) and is referred to as the plastic response of fitness on damage (Abrahamson and Weis 1997). The slope of this regression is the estimate of tolerance; a shallow slope indicates low sensitivity to damage, or high tolerance, whereas a large negative slope indicates a high sensitivity to damage, or low tolerance. A positive slope indicates overcompensation. For our analyses, we took the difference in the average, relative fitness of each maternal line from two treatment applications (or  $W_d - W_u$ ) as our estimate of tolerance, following established methods (Baucom and Mauricio 2004; Tiffin and Rausher 1999).

Maternal line values of tolerance were then averaged for each population and species for performing the spatial autocorrelation analysis.

### ‘Hotspots’ analysis

To test for positive or negative spatial autocorrelation of tolerance over the Southeast, we calculated Moran’s  $I$  (Moran 1950) over mutually exclusive spatially lagged distance classes. A high positive value of Moran’s  $I$  would indicate the spatial clustering of similar values, whereas a high negative value indicates dissimilar values. Unlike other studies that assess if populations spatially aggregate for high or low levels of a trait value (Brodie Jr et al. 2002) this analysis determines if populations aggregate for similar or dissimilar values of tolerance, regardless of the level of tolerance. The higher the value of Moran’s  $I$ , the more the observation is similar (positive) or dissimilar (negative) to its neighbors. The analyses were performed for each species separately within either the Coastal Plain or the Cumberland Plateau. We performed the analyses per each region because populations within each of the regions were widely separated from one another (Fig. 1), and Moran’s  $I$  values are affected by discontinuities in sampling patterns (Epperson and Li 1996). The first quartile of nearest-neighbor statistics was estimated in the program ROOKCASE (Sawada 1999) for each species within each region and was used to determine the size of the lag distances. For example, if the first quartile of nearest-neighbor statistics was 7,000 m, each lag distance was set at 7,000 m and a global Moran’s  $I$  statistic was calculated for each distance. Significance in the lag distance classes was tested in ROOKCASE by using 1,000 Monte Carlo permutations of Moran’s  $I$ , and a 95% confidence envelope was generated from the permutations. Values of Moran’s  $I$  within 95% confidence envelope represent cases where the null hypothesis of no spatial autocorrelation cannot be rejected. Values outside of this range indicate significant spatial autocorrelation, or evidence of either ‘hotspots’ or ‘coldspots’ of tolerance. To take into account multiple tests over the lag distances, a manual Bonferroni correction was used to determine if the correlograms were globally significant (M. Sawada, personal communication).

## Results

### Genetic and geographical variation for tolerance

We first determined whether genetic variation existed for tolerance among maternal lines within populations of both species. The maternal line by treatment interaction term was marginally significant for *I. purpurea* (Maternal Line  $\times$  Treatment  $Z = 1.51$ ,  $P = 0.06$ ; Table 2), but not significant for *I. hederacea* (Maternal Line  $\times$  Treatment estimate = 0; Table 3), indicating that the maternal individuals of *I. hederacea* did not differ in the regression of fitness on the treatment environments, and that genetic variation for tolerance was present only within *I. purpurea* populations.

We next determined if variation existed among populations and regions for both species. In the same nested mixed model, the population by treatment and region by treatment interactions assessed if there was variation among populations and regions for tolerance, respectively. The population by treatment interaction term was



**Table 2** Results of REML partitioning of variation for square-root transformed relative fitness in a full-mixed model for *I. purpurea*

Source of variation	Variance estimate	Z	P-value
Random effects			
Maternal line (population region)	0.0000		
Population (region)	0.0011 ± 0.003	0.33	0.371
Maternal line × treatment	0.0077 ± 0.005	1.51	0.066
Population × treatment	0.0055 ± 0.004	1.22	0.112
Residual	0.1293 ± 0.007		
Fixed effects			
	<i>df</i> <sup>a</sup>	F-value	P-value
Treatment	1, 21.2	1078.89	<0.0001
Region	1, 22.6	8.49	0.007
Region × treatment	1, 21.2	6.76	0.016

Each variance component is followed by one SE. P-values were determined from Wald Z-tests

<sup>a</sup> The Satterthwaite method was used to determine *df*

**Table 3** Results of REML partitioning of variation for square-root transformed relative fitness in a full-mixed model for *I. hederacea*

Source of variation	Variance estimate	Z	P-value
Random effects			
Maternal line (population region)	0.0069 ± 0.01	1.24	0.107
Population (region)	0.0000		
Maternal line × treatment	0.0000		
Population × treatment	0.0279 ± 0.01	2.58	0.005
Residual	0.1386 ± 0.01		
Fixed effects			
	<i>df</i> <sup>a</sup>	F-value	P-value
Treatment	1, 24.1	250.89	<0.0001
Region	1, 27.2	0.00	0.956
Region × treatment	1, 24.1	0.18	0.677

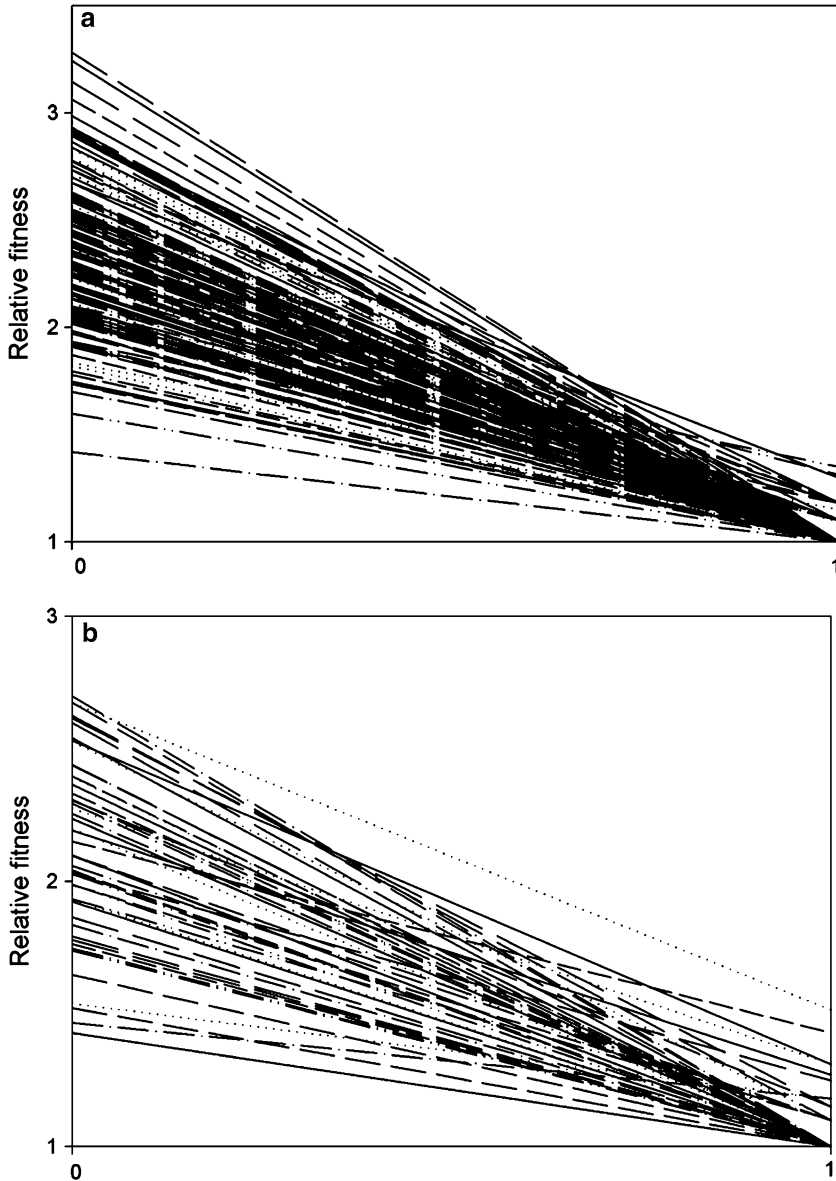
Each variance component is followed by one SE. P-values were determined from Wald Z-tests

<sup>a</sup> The Satterthwaite method was used to determine *df*

significant for *I. hederacea* (Population × Treatment  $Z = 2.58$ ,  $P = 0.004$ ; Table 3), but not *I. purpurea* (Population × Treatment  $Z = 1.37$ ,  $P = 0.112$ ; Table 2) indicating that the populations of only *I. hederacea* exhibited variation over geography for tolerance (see Fig. 2 for a graphical representation of tolerance). At the regional level *I. purpurea* exhibited significant variation among regions for tolerance (Region × Treatment  $F = 6.76$ ,  $P = 0.016$ ; Table 2) but *I. hederacea* did not (Region × Treatment  $F = 0.18$ ,  $P = 0.678$ ; Table 3).

#### Variation among species and regions

Overall, the two species are exhibiting marginally different levels of tolerance in the Southeast (average tolerance, *I. purpurea*:  $-1.22$ , *I. hederacea*:  $-1.11$ ,  $F = 2.24$ ,  $P = 0.06$ , Table 4; Fig. 3). In addition, there was a significant species by treatment by region interaction ( $F = 9.25$ ,  $P = 0.003$ , Table 4), suggesting that the species are exhibiting different levels of tolerance in the different regions.



**Fig. 2** Square-root transformed relative fitness in response to glyphosate application, averaged for each maternal line for **(a)** *I. purpurea* and **(b)** *I. hederacea*. A '0' on the x-axis represents the no herbicide environment and a '1' corresponds to the presence of herbicide

#### 'Hotspots' and 'coldspots' of tolerance in the Southeast

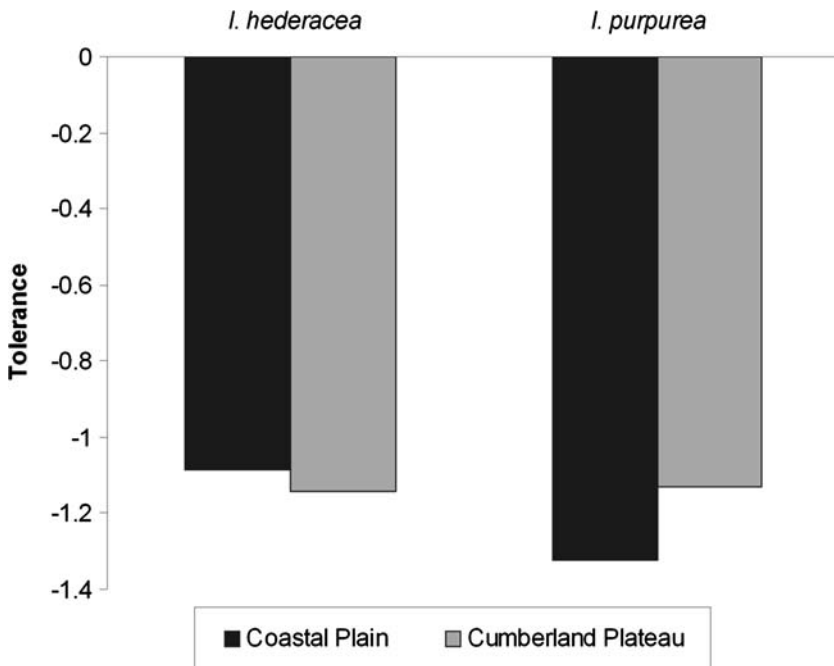
Significant positive spatial autocorrelation was found among *I. purpurea* populations from the Coastal Plain (Fig. 4a) at the first lag distance tested (Moran's  $I = 2.635$ ,  $P = 0.004$ ), indicating a significant hotspot of tolerance on a local scale. At the third lag distance, or 21 km, a significant negative association was found among

**Table 4** Results of REML partitioning of variation for square-root transformed relative fitness in a full-mixed model with both species included

Source of variation	Variance estimate	Z	P-value
<b>Random effects</b>			
Maternal line (population region)	0.0025 ± 0.004	0.66	0.255
Population (region)	0.00		
Maternal line × treatment	0.0068 ± 0.005	1.23	0.110
Population × treatment	0.0097 ± 0.003	2.80	0.003
Residual	0.1337 ± 0.006		
<b>Fixed effects</b>			
	<i>df</i> <sup>a</sup>	<i>F</i> -value	<i>P</i> -value
Treatment	1, 43.6	886.23	<0.0001
Species	1, 262	0.49	0.483
Region	1, 45.9	3.27	0.077
Species × region	1, 262	7.16	0.008
Species × treatment	1, 260	3.56	0.060
Region × treatment	1, 43.6	1.67	0.203
Species × region × treatment	1, 260	9.25	0.003

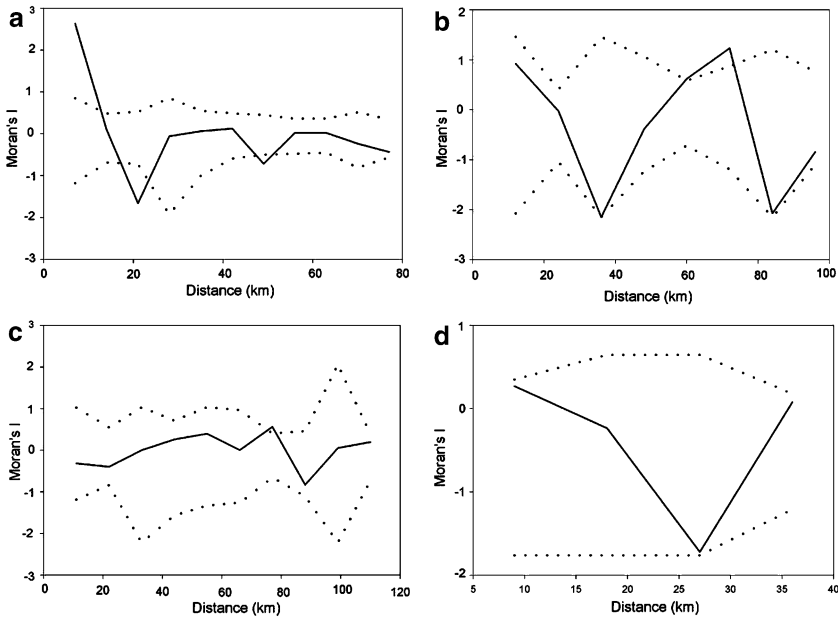
Each variance component is followed by one SE. *P*-Values were determined from Wald *Z*-tests

<sup>a</sup> The Satterthwaite method was used to determine *df*



**Fig. 3** Differences in the level of tolerance between regions within species using least square means to estimate tolerance. *I. purpurea* exhibited a significant region by treatment interaction (*P* = 0.02) in the mixed-model analysis of variance (Table 2)

populations tested, meaning that, at greater distances from the central reference point, populations were significantly dissimilar for their level of tolerance (Moran’s *I* = -1.656, *P* = 0.001). This correlogram was found to be globally significant after



**Fig. 4** Correlograms of Moran's  $I$  for (a) *I. purpurea* populations within the Coastal Plain, (b) *I. purpurea* populations within the Cumberland Plateau, (c) *I. hederacea* populations within the Coastal Plain, and (d) *I. hederacea* populations from the Cumberland Plateau. Values outside of the 95% confidence intervals indicate significant levels of either positive or negative spatial autocorrelation. About 1,000 Monte Carlo permutation tests were performed to construct the confidence intervals

using a manual Bonferroni correction to take multiple tests into account ( $P = 0.005$ ). A significant negative Moran's  $I$  was found at the third lag distance from *I. purpurea* populations in the Cumberland Plateau (Fig. 4b), and a positive value of Moran's  $I$  was found at the sixth lag distance; however, this correlogram was not globally significant, meaning that this region did not exhibit spatial autocorrelation for tolerance values. In addition, *I. hederacea* populations did not show any significant spatial autocorrelations from either region (Fig. 4c, d), after applying the Bonferroni correction to the correlograms. For at least one region of the Southeast, however, evidence of significant hotspots and coldspots were found, meaning that populations within sites are clumped spatially for their level of tolerance, and that a mosaic of tolerance exists for one species of morning glory in the Southeast.

## Discussion

### Geographic patterns of phenotypic variation

There is growing data to suggest that populations vary according to defensive traits such as resistance to predators and pathogens (Burdon and Thrall 1999; Brodie Jr et al. 2002), and level of resistance to herbicides (Warwick 1991). Our results support this conclusion on multiple levels of geography in that we find evidence for within- and among-population variation, as well as among-region variation in the level of

tolerance in assays of two morning glory species. Specifically, we found evidence of a significant maternal line effect, or within-population variation in *I. purpurea* for tolerance to glyphosate. This was expected, given that within-population variation for tolerance has previously been documented in one population from the Southeast (Baucom and Mauricio 2004).

There was no evidence, however, of within-population variation for tolerance in *I. hederacea*, but we did detect among-population variation for this species. The lack of within-population variation could be due to a lack of statistical power, given that we used only three replicates per maternal line in the experiment. A retrospective power analysis gave a power value of 0.61 for the maternal line by treatment interaction term in *I. hederacea*. The pattern of no within-population variation and the presence of among-population variation could also be attributed to this species' highly selfing mating system. This pattern is typical of predominantly selfing species, which maintain half of their neutral genetic variation among subpopulations (Hamrick and Godt 1996), and could occur given population establishment by a single or few individuals tolerant to glyphosate. Those individuals would predominate genetically within populations, especially given selection by glyphosate, and lead to more among- than within-population variation.

It is also relevant to this scenario that we did not find evidence of among-population variation in the primarily outcrossing species, *I. purpurea*. Gene flow between populations could lead to a similar level of tolerance among all populations, as tolerance alleles could be dispersed through either pollen or seed dispersal. Pollen dispersal is the more likely candidate, because seed dispersal as the primary mechanism of gene flow would lead to similar spatial patterns between the species, since seeds of both species are gravity dispersed and are equally likely to be moved by farm machinery. That *I. hederacea* maintains genetic variation for tolerance among populations suggests that pollen movement acts to effectively homogenize trait values among populations of *I. purpurea*.

Although we found evidence of among- population variation for *I. hederacea*, there appeared to be no among-region variation in tolerance for this species, as was found in *I. purpurea*. This result could again be due to a lack of power in our analyses given that we were able to sample only six populations of *I. hederacea* from the Cumberland Plateau and 11 populations from the Coastal Plain (power = 0.13). If the difference between the two species in how they distribute their respective level of genetic variation for tolerance is a biological phenomenon, it could potentially be due to differences in their mating systems. Gene flow among populations within regions in *I. purpurea* could homogenize trait values, and lead to one region being more tolerant than the other. Likewise, the variation among regions in *I. purpurea* in the level of tolerance could be due to random processes, such as drift in the process of population establishment or selection on a trait correlated to tolerance. Adaptation to other abiotic or biotic factors not examined by this common garden study could also potentially explain variation among regions in tolerance.

Alternatively, variation in farmer's practices among populations and regions could be responsible for the trait differentiation that the two species exhibit. Our initial expectation was that a higher rate of glyphosate use or more episodes of spraying would explain spatial variation in the level of tolerance. Unfortunately, censuses of the farmers provided no evidence for differences in the rate of glyphosate used, as almost all farmers or extension agents indicated the same rate of spray. In addition, there were no clear indications given of differences in glyphosate

use between the regions; however, variation in crop rotations and the number of times per season glyphosate was sprayed existed among all farmers censused. Given that the spraying histories could not be clearly indicated as the source of the spatial variation, we chose to consider the following logic: if selection on tolerance by glyphosate produced the apparent differentiation among regions in *I. purpurea*, then the amount of adaptive genetic variation for this trait should be greater than that of neutral genetic variation. A measure of the regional quantitative trait variation in tolerance, or a ' $Q_{st}$ ', was estimated as 0.691, following the method of Spitze (1993). Although we do not have an estimate of  $F_{st}$ , or measure of neutral genetic variation for this particular species, the  $F_{st}$  of species with a similar mating system and seed dispersal mechanism to *I. purpurea* has been shown to be  $0.248 \pm 0.19$  (Hamrick and Godt 1989, 1996). This estimate is much lower than our estimate of quantitative trait variation, which suggests that directional selection underlies the variation among regions in *I. purpurea*. This is circumstantial evidence for selection producing the apparent pattern of trait variation in this study, and it highlights the need for further study of this system. Reciprocal transplants of the species among the Coastal Plain and Cumberland Plateau, as well as monitoring patterns of herbicide use in the future may help to clarify the extent to which selection by the herbicide spraying regime has led to the spatial patterns of trait variation.

#### Variation among species and regions

We found only weak evidence that one species was more tolerant to glyphosate than the other, with *I. hederacea* appearing the more tolerant. However, we did find evidence that the species are responding differently to glyphosate in the different ecological regions. Our evidence for this phenomenon comes from a three-way interaction term in the ANOVA, which makes the biological interpretation difficult. If our interpretation is correct, then in the Coastal Plain, *I. hederacea* appears to be more tolerant than *I. purpurea*, yet this relationship does not hold in the Cumberland Plateau. It was expected that *I. hederacea* would be more tolerant to glyphosate application than *I. purpurea*, at least based on interviews with farmers from the Coastal Plain, who suggested that *I. hederacea* appeared to respond less to glyphosate spray. However, the same relationship between the species was not found within the Cumberland Plateau. This finding underscores the importance of considering the ecological region from which experimental species are drawn before management decisions are made. Using the response of one species to glyphosate in one ecological region as the model for management decisions over wide areas would make controlling the evolution of tolerance in crops largely unpredictable.

#### The geographic mosaic

We found evidence of tolerance 'hotspots' and 'coldspots,' or spatial autocorrelation of tolerance within one region of the Southeast in *I. purpurea*. This data provides evidence that tolerance can cluster significantly on a very local scale, or within 7 km. On average, *I. purpurea* populations were 25 km from one another in the Coastal Plain. Finding a positive autocorrelation within 7 km suggests that the populations that exhibited similar levels of tolerance have likely either been exposed to similar selective regimes in the past, or are close enough for gene flow to exert a homogenizing effect. Either scenario is equally plausible: the same farmer will often use a

similar herbicide regime to control weeds in different fields, and, alternatively, morning glory seeds are reported to likely be transported across fields by farm equipment.

This aggregation of similar or dissimilar levels of tolerance was not found in either species within the Cumberland Plateau or in either region for *I. hederacea*. Taken at face value, it is apparent that the populations comprising these designations are independent units undergoing distinct evolutionary forces and responding on their own evolutionary trajectory. This lack of trait value ‘clumping’ suggests that the populations sampled closest to one another were not part of a metapopulation, or a larger population connected by gene flow. Alternatively, it is entirely possible that metapopulations of *I. hederacea* exist at a finer scale of sampling than was used in the present study. Finding significant spatial autocorrelation in one species in the Southeast suggests that both evolutionary biologists and weed managers should consider more than just the single population when studying the evolutionary dynamics of plant traits, especially those traits that confer high fitness advantage such as tolerance to herbicide.

## Conclusion

The two species of morning glory investigated in this study are partitioning their respective levels of genetic variation for tolerance to glyphosate in alternate ways. *I. purpurea* shows marginally significant variation within populations as well as differentiation among ecological regions for tolerance. *I. hederacea* shows differentiation in tolerance levels among populations only. We suggest that the spatial scale of variation differs between the species largely due to their differing mating systems. *I. purpurea* employs a mixed-mating strategy, but is largely outcrossing, whereas *I. hederacea* is almost completely selfing.

Spatial and geographic patterns of genetic variation have long been of interest to genetics since spatial structuring can influence mating system dynamics, gene flow and patterns of selection (Endler 1977). We found evidence of positive and negative spatial autocorrelation, suggesting that populations found closer to one another exhibit similar levels of tolerance whereas populations found further apart exhibit differing levels of tolerance. In sum, our data suggests that the continued evolution of tolerance to glyphosate is, in large part, dependent on the individual species, agricultural field, region in question, as well as units in the geographical hierarchy that often are ignored: the potential for population aggregation of trait values. Continued examination of populations of the two species over time will help to clarify the nature of trait variation among them.

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