

Genetic Differentiation and Phenotypic Plasticity of Forest Herbaceous Species in Iowa, Central United States

Emily A. Altrichter, Catherine M. Mabry, Janette R. Thompson and Randall K. Kolka

ABSTRACT

The forest herbaceous layer provides important ecosystem services in the central United States. However, human impacts have caused declines of many of these species. Restoration of this layer is uncommon in temperate forests, so best practices are not yet established. There has been widespread concern about negative outcomes (for example, failure due to genetic swamping or outbreeding depression) when plant material is transferred beyond a local scale. Current practice is to use local sources under the assumption that they are optimal genotypes for the site. However, few local sources are available for many species. We examined genetic variability and phenotypic plasticity by comparing performance of local and non-local populations (from sites approximately 250 km apart) of six forest herbaceous species. We used a common garden study to test for genetic differences in plant traits, and a field study to test for phenotypic plasticity. Based on the common garden we found genetic differences between local and non-local populations for each species. Trait differences we observed in greenhouse trials we also detected in the field in the first year. However, these differences diminished in the second year of the field study and we did not detect them in the four species measured in the third year. This provided evidence that phenotypic plasticity was operating, as plant characters responded plastically to local conditions. We found no evidence that local plants consistently outperformed non-local plants. These results suggest less need for strict adherence to locally sourced seeds or transplants.

Keywords: forest perennial herbaceous species restoration, forest understory, local ecotypes, restoration plant sourcing

🌿 Restoration Recap 🌿

- Forests and the forest herbaceous layer provide valuable ecosystem services. The restoration of key forest species can increase the capacity to provide such services.
- Secondary woods and native remnant forests that have been subject to human disturbances such as cattle grazing, intense herbivory by deer, or recreational use by humans often degrade herbaceous layer plant communities. These become dominated by generalist species and are missing forest specialists.
- Herbaceous layer species can be successfully restored to forests and have relatively high survival rates, good vegetative growth, and reproductive success.
- Phenotypic plasticity may allow both local and non-local genotypes to adapt to local environments, suggesting that sourcing zones could include broader areas than previously estimated.

The forest herbaceous layer, while representing less than one percent of the forest biomass, can contain 90 percent or more of the forest species diversity. This layer is a critical component of functional and diverse forest ecosystems. For example, the classic Hubbard Brook experiments in central New Hampshire emphasized the functional

importance of the understory layer and introduced the vernal dam hypothesis, which suggests that spring-growing understory plants take up significant quantities of nutrients, particularly nitrogen and phosphorus, decreasing nutrient pollution in streams early in the growing season (Muller and Bormann 1976). The importance of nutrient uptake by this layer has been confirmed in numerous additional studies (Blank et al. 1980, Peterson and Rolfe 1982, Tremblay and Larocque 2001, Gerken Golay et al. 2013b, Gerken Golay et al. 2016). In addition, these plants

perform a host of other functions in the forest, including holding soil, governing competitive interactions, linking the overstory to understory seedling dynamics, and supporting a wide range of pollinating insects (Gilliam 2007, Hanula et al. 2016).

Most remnant forests in the central United States region have undergone vegetation change and a decline in diversity. These changes are typically the direct or indirect result of human activity, including cattle grazing, deer overabundance, nonnative earth worms, nonnative plant introductions, and heavy recreational use in urban and suburban areas (e.g., Gibson et al. 2000, Mabry 2002, Rooney et al. 2004, Andrés-Abellán et al. 2005, Nuzzo et al. 2009, Cameron et al. 2015). Forests that have been subjected to these forms of disturbance, as well as new secondary forests, have fewer native herbaceous species than are present in preserved forests, and instead have understories composed of bare ground or herbaceous plant communities dominated by generalist and invasive species (Mabry 2002, Rooney et al. 2004, Gerken et al. 2010, Gerken Golay et al. 2013b). Consequently, many woodlands across this broad region are unlikely to have a full complement of native understory species. Because many woodland species have limited dispersal potential (Mabry 2002), we cannot rely on succession or natural dispersal to restore many species to degraded sites. This combination of human disturbance and dispersal limitation suggests that an active restoration program is needed. However, restoration of the herbaceous layer in the central U.S., and in temperate forests more generally, is uncommon, and best practices have not yet been established.

One of the primary debates in restoration concerns the identity of genetically and geographically appropriate plant material. Genetic differentiation and phenotypic plasticity are two phenomena that govern restoration protocols and prevailing opinions on plant material sources. The first, genetic (or local) differentiation, is based on the idea that populations adapt to local environmental conditions, resulting in genetically distinct populations within species, e.g., ecotypes that perform best under those local conditions (Linhart and Grant 1996, Joshi et al. 2001, Hufford and Mazer 2003). In addition, there is evidence that many forest herbaceous species exhibit genetic differentiation (as evidenced when grown in a common garden) at a regional scale of about 250 kilometers (Gerken-Golay 2013a, Mabry 2017).

The second, phenotypic plasticity, occurs when plants change morphologically in response to year-to-year changes in local environmental conditions. If a plant trait is plastic, a single genotype can produce a range of phenotypes in response to different environmental conditions (Bradshaw 1965). In doing so, plasticity may mask genetic differences (Schlichting 1986, Sultan 2000). Phenotypic plasticity has been widely studied in the theoretical literature, but there are few empirical studies that address it

in the restoration literature (however, see Gerken Golay et al. 2013a).

Many practitioners assume that local plant material performs better than non-local sources. We sought to address this assumption about plant sources for restoration by comparing morphological and reproductive traits of transplanted local and non-local populations of six forest herbaceous perennial species. This is an important question because few local sources are available for many species of interest for restoration in the central U.S., so they must be obtained from broader collection zones (Altrichter et al. 2017). Our goal was to determine the extent to which genetic differentiation and/or phenotypic plasticity are occurring among these plants.

We used a greenhouse (common garden) experiment to determine whether there were genetically based differences in vegetative and reproductive traits between local and non-local populations of these species. We also conducted a separate field study over three field seasons to test whether any differences observed in the greenhouse were also consistently expressed when populations were transplanted into natural forest ecosystems. Specifically, we explored the following research questions: 1) Are genetic differences expressed morphologically between local and non-local populations of woodland herbaceous perennial species? and 2) To what degree are those differences obscured by phenotypic plasticity in the field? Morphological differences observed in the greenhouse (common garden) can be attributed to a genetic basis, while differences in expression between the common garden and field, and in the field from year to year, can be attributed to phenotypic plasticity. We also recorded percent survival for both studies because it is important to document this in the restoration literature (Bjureke et al. 2011).

Methods

Study Area

Our study area, central Iowa, U.S.A., is located at the transition zone between the eastern deciduous forest that characterizes the Northeast and Midwestern United States, and the tallgrass prairie region. The modern forest community in Iowa is dominated by *Quercus alba* (white oak), *Q. macrocarpa* (bur oak), *Q. rubra* (red oak), and *Q. velutina* (black oak), *Carya ovata* (shagbark hickory), *Acer nigrum* (black maple), *Tilia americana* (American basswood) and *Prunus serotina* (black cherry) (van der Linden and Farrar 2011). The landscape of Iowa was historically a mosaic of tallgrass prairie, prairie pothole wetlands, and upland and riparian forests, but much of Iowa's arable land has been converted to agriculture. Approximately 78% of Iowa's land area is used for intensive row-crop farming or pasture (Gallant et al. 2011). Remnant forests in this landscape tend to be

located on land that is hilly, or as gallery forests along rivers and streams (Thompson 1992).

Species Selection, Transplant Sources and Collection

We chose to study six herbaceous perennial species: *Asarum canadense* (wild ginger), *Hydrophyllum virginianum* (Virginia waterleaf), *Mertensia virginica* (Virginia bluebells), *Polygonum virginianum* (jumpseed), *Ranunculus hispidus* (bristly buttercup) and *Solidago flexicaulis* (zigzag goldenrod). We selected *A. canadense* and *H. virginianum* because a previous pilot study suggested that they exhibit both genetic differentiation and plastic responses to variation in the field (Gerken et al. 2013a). We chose the remaining four species due to their high potential for production of biomass (and therefore nutrient capture, e.g., Mabry et al. 2008, Gerken Golay et al. 2016), as well as phenological growth patterns that span the growing season (Gerken Golay et al. 2016).

All six species are long-lived perennials, and are insect pollinated (outcrossed). All six also can be ubiquitous in remnant forests but are often missing from new secondary woods and sites that have been subject to long-term disturbance (Mabry 2002, Mottl et al. 2008).

We compared plants between our central Iowa study area (hereafter local plants), and northeast Iowa (hereafter non-local plants). We chose this comparison based on previous research that provided evidence that there are genetically based differences in trait expression between the two areas (Gerken Golay et al. 2013a, Mabry 2017). This choice also conforms to three collection zones adhered to by the Iowa Ecotype Project, wherein the state is divided into three roughly equal zones: south, central, and north (Houseal and Smith 2000). Our local plants were from the central zone and non-local plants were from the north zone.

Local plants were collected from four forests on both public and private land in Story and Boone counties in central Iowa. We collected plants of the same species from sites less than 1 kilometer apart. This area is part of the Des Moines Lobe landform region. It is the youngest landform in the state, last glaciated in 12,000 to 14,000 BP. It was historically composed of prairie potholes, prairies, savannas, and woodlands, many of them along the streams and rivers (Prior 1991). Average annual temperature in this area is approximately 9.7°C, and average annual precipitation is 90.9 cm.

Non-local plants were collected from Yellow River State Forest (YRSF), Allamakee County, Iowa, approximately 250 km away from the central Iowa collection area. Within species, plants were also collected from sites than 1 kilometer apart. Northeast Iowa is part of the Paleozoic Plateau, also historically known as the Driftless Area. It has not been recently glaciated and is known for its steep topography and deep valleys, and historically included and still includes

abundant upland woods dominated by the genera *Quercus* (oak) and *Carya* (hickory) (Prior 1991, van der Linden and Farrar 2011). The average annual temperature for YRSF is 7.5°C, with average annual precipitation of 91.7 cm. We excavated individual plants of each species shortly after plant shoots emerged in late April to early May, 2014.

All plants were collected from historical remnant forests. We carefully monitored the timing of collection to ensure that plant material from both the central and northeast Iowa sources were uniform in size at the time of collection. Because it is impossible to know the chronological age of these long-lived plants, we chose plants in uniform developmental stages. For example, when we collected *H. virginianum*, we chose plants with 3–5 leaves for both the central and northeast Iowa sources, and selected plants of average size, avoiding larger or smaller individuals. We carefully excavated individual plants to obtain intact root systems, wrapped the roots in wet paper towels, placed them in plastic bags and transported them to the greenhouse or field sites in coolers. Plants were cold-stored for up to four days until we planted them in the greenhouse and at field sites.

Greenhouse Study

The greenhouse study was conducted as a separate experiment to ascertain genetic differences between plant sources. Because the greenhouse is a uniform environment, morphological differences observed there can be assumed to be genetically based. We planted 16 local and 16 non-local plants for a total of 32 plants of each species in 15.2-cm diameter pots using Sunshine LCI mix growing medium. The pots were placed on benches, randomized, and rotated regularly to prevent bench effects. Plants were watered twice per day and sprayed for pests as needed. Plant development was monitored from April or May to October, 2014.

Field Study

We established plots in three urban forest areas in Ames, Story County, Iowa that were relatively free of invasive species and had few or none of the study species already present. The three forest areas ranged in size from 16 to 183 ha. Two of the sites were in the City of Ames Parks system, and the third site was under the jurisdiction of the Ames YWCA and Iowa State University. All three sites have been managed to provide public areas for passive recreation and there is no recent history of silvicultural management of forest vegetation.

We planted five plots in each of the three sites. Each plot consisted of 12 quadrats, with each quadrat comprised of four plants. Each quadrat represented one of the six species/local versus non-local source combinations, for a total of 15 plants per field site for each combination. The corner of each plot was marked with a plastic survey stake, and

GPS coordinates were recorded for each plot location. Each plant was individually marked with flagging to ensure that we measured the same plant each year. We watered them once at the time of planting to establish soil-root contact. Existing vegetation (other than individuals of the study species which were removed if present) was left in the field plots to mimic natural competitive conditions.

Data Collection and Analysis

Greenhouse plants were measured in 2014. The field study plants were measured in 2014 and 2015. In addition, in 2016, at each of the three sites we measured a subset of traits for four of the six species at three of the five plots. We chose to re-sample only the four spring-growing species due to time constraints in the fall that precluded measuring the remaining two species and plots.

For plants in both the greenhouse and field plantings, for each year we measured a set of vegetative characteristics which varied somewhat according to species to compare local and non-local sources (within years). In both the greenhouse and field study growth was sufficient to obtain data on flower production for four of the six species. We chose characters that would be important for growth, establishment, and vigor (e.g., Gerken Golay et al. 2013a). For example, vegetative traits like stem diameter, leaf size, and tiller number indicate vegetative vigor and ability to take up space and resources, while reproductive traits like flower number and seed number indicate the potential to spread and establish new populations.

We used one-way ANOVA to test for differences in measured traits between the local and non-local plants in the greenhouse, with location of the source population as the independent variable, and vegetative and reproductive traits as the response variables. In the field study we used two-way ANOVA to test for these differences within each year, with field site treated as a blocking factor and location of source population as the independent variable. Plasticity was not measured directly but was inferred when trait values changed over time in response to changing environmental conditions (Bazzaz and Sultan 1987).

Plots were sufficiently spaced to be independent (e.g., at least 50 m between plots) and were the sampling unit. Plants within quadrats were considered subsamples and

were averaged, yielding five sampling units per site, times three sites, or fifteen sampling units. No transformations were made to the data. We set the *p*-value for significance at ≥ 0.05 . Sites (blocks) were not significant for a majority of the species and traits across all three years, and for simplicity we did not present these results in the tables. Survival was recorded but not analyzed because differences in survival between local and non-local sources was not hypothesized as part of this study. Statistical analyses were conducted using Data Desk Version 7 (Data Description, Inc., Ithaca, New York).

Results

Greenhouse Study

Bare-root plants from both local and non-local populations had very high survival rates (88–100%) in the greenhouse (common garden) experiment (Table 1). Morphological differences between local and non-local populations in the greenhouse demonstrated that there was a genetic basis for differences for one or more traits for all six species (Table 2). For some species and traits local genotypes were more robust, and in others the non-local plants were more robust (Table 2). For example, the local *M. virginica* had much greater leaf and flower numbers compared to the non-local; however, the non-local plants of this species had greater leaf area (Table 2). In another example, local *H. virginianum* had a greater number of leaves, but the non-local plants had a higher leaf area (Table 2).

Field Study

Average survival rates were 78% for local seedlings and 81% for non-local plants at the end of 2015. Survival rates generally varied between 72% and 92% for local plants and between 70% and 97% for non-local plants (the low survival rate for *S. flexicaulis* was due to late-season flood damage in 2015 on one set of field plots) (Table 1). We also noted recruitment (establishment of new plants) in the second field season for *A. canadense*, *R. hispidus*, and *S. flexicaulis*.

Generally, the differences we observed in the greenhouse we also observed the first year of the field study (2014)

Table 1. Percent survival of local and non-local transplants of six species included in this study grown in the Iowa State University Forestry Greenhouse (measured in 2014) and at three forest field sites (measured in 2015) in Ames, Iowa.

Species		Greenhouse		Field sites	
		Local	Non-local	Local	Non-local
<i>Asarum canadense</i>	Wild ginger	88%	100%	72%	70%
<i>Hydrophyllum virginianum</i>	Virginia waterleaf	100%	100%	85%	97%
<i>Mertensia virginica</i>	Virginia bluebells	100%	100%	83%	92%
<i>Polygonum virginianum</i>	Jumpseed	100%	100%	92%	80%
<i>Ranunculus hispidus</i>	Bristly buttercup	100%	100%	82%	73%
<i>Solidago flexicaulis</i>	Zigzag goldenrod	94%	100%	40%	78%

Table 2. Means and one-way ANOVA results for traits measured for six perennial herbaceous understory species transplanted to the State University Forestry Greenhouse in Ames, Iowa, in 2014. Asterisks indicate differences in traits between local and non-local plants.

Species/trait	Local mean	Non-local mean	MSE population	F value	p-value
<i>Asarum canadense</i>					
Fruit number	0.7	0.8	0.07	F _{1, 28} 0.3	0.603
Leaf area (cm ²) *	49.3	79.6	6842.00	13.5	0.001
Leaf number	2.0	2.0	0.00	0.0	1.000
Stem diameter (cm) *	0.4	0.5	0.08	20.7	< 0.001
<i>Hydrophyllum virginianum</i>					
Flower number	32.1	23.6	320.3	F _{1, 32} 1.1	0.312
Leaf area (cm ²) *	134.4	199.8	36256.2	8.6	0.006
Leaf number *	8.7	6.1	59.9	10.6	0.002
<i>Mertensia virginica</i>					
Flower number *	29.8	4.1	5253.1	F _{1, 30} 22.8	< 0.001
Height (cm)	21.7	19.0	60.2	2.4	0.132
Leaf area (cm ²) *	36.1	62.4	5561.3	10.0	0.004
Leaf number *	20.1	5.7	1653.1	34.4	< 0.001
Stem diameter (cm)	0.41	0.4	0.03	3.0	0.093
<i>Polygonum virginianum</i>					
Height (cm) *	69.3	56.2	1335.4	F _{1, 30} 19.0	< 0.001
Leaf area (cm ²) *	180.5	206.0	5225.2	8.6	0.006
Leaf number *	48.7	34.8	1554.0	20.1	< 0.001
Stem diameter (cm)	0.6	0.6	0.0	0.3	0.624
<i>Ranunculus hispidus</i>					
Leaf number *	6.4	22.2	1984.5	F _{1, 30} 6.7	0.014
<i>Solidago flexicaulis</i>					
Height *	44.9	64.2	3066.3	F _{1, 31} 11.2	0.002
Leaf area *	75.8	105.2	7056.9	9.1	0.005
Leaf number *	23.9	50.1	5583.0	15.5	0.014
Seed head number	26.7	19.8	382.1	3.2	0.085
Stem diameter *	0.31	0.4	0.1	22.0	< 0.001

(Tables 2 and 3). The exceptions were for leaf area for *H. virginianum*, height and leaf number for *P. virginianum*, and stem diameter for *S. flexicaulis*. In addition, there were three traits that differed for plants in the field at the end of the first year that we did not observe in the greenhouse: fruit number for *A. canadense*, flower number for *H. virginianum*, and height for *M. virginica*.

In the second year of the field study, there were only six differences observed between local and non-local plants across the six species, compared to 16 in the first year of the field study. Two of the six differences observed were traits that were measured the previous year, but with no evidence of difference (Tables 3 and 4).

The additional data collected in 2016 supported the trend of fewer differences observed in the field over time (Table 5). No statistical differences were observed in traits between the local and non-local populations for the four species measured, even for traits that had exhibited strong differences earlier, such as leaf area in *A. canadense*, flower number in *H. virginianum*, and leaf number in *M. virginica* (Tables 3 and 5). These results reflect the very low effect sizes observed for all traits and species with the possible exception of flower number in *M. virginica*.

Finally, similar to the greenhouse results, there was no evidence from the field study that local populations or non-local populations were consistently more robust than the other. For example, in the first year of the field study non-local *A. canadense* and *S. flexicaulis* outperformed the local plants for all traits that differed. In contrast, local *H. virginianum* outperformed non-local plants for both traits that differed, and local *M. virginica* outperformed non-local plants for three of the four traits that differed (Table 3).

Discussion

In this study, we evaluated survival, genetic differentiation, and phenotypic plasticity for local and non-local populations of six forest herbaceous species. We observed high survival rates in the greenhouse study and field study for both populations.

Based on previous studies (e.g., Gerken Golay et al. 2013a, Mabry 2017) we expected to find genetic differences expressed morphologically between the two source populations for some of the six species we studied. In the greenhouse (common garden), we found that each species

Table 3. Means (2014) and two-way ANOVA results (block results not shown) for traits measured for six perennial herbaceous understory species transplanted to three field sites in Ames, Iowa. Asterisks indicate differences in traits between local and non-local plants.

Species/trait	Local mean	Non-local mean	MSE population	F _{1, 26}	p-value
<i>Asarum canadense</i>					
Fruit number *	0.2	0.4	0.4	6.3	0.018
Leaf area (cm ²) *	38.5	57.1	2607.6	25.7	< 0.001
Leaf number	1.8	1.9	0.1	3.4	0.078
Stem diameter (cm) *	0.3	0.4	0.04	51.1	< 0.001
<i>Hydrophyllum virginianum</i>					
Flower number *	17.6	6.1	994.8	19.8	< 0.001
Leaf area (cm ²)	49.1	54.1	188.2	1.3	0.266
Leaf number *	3.4	2.2	10.3	20.9	< 0.001
<i>Mertensia virginica</i>					
Flower number *	24.4	3.5	59.1	6.9	< 0.001
Height (cm) *	19.5	16.7	3276.1	34.1	< 0.001
Leaf area (cm ²) *	14.5	30.2	1842.4	43.0	< 0.001
Leaf number *	11.8	6.0	256.7	31.1	< 0.001
Stem diameter (cm)	0.4	0.4	0.0	0.7	0.408
<i>Polygonum virginianum</i>					
Height (cm)	24.4	23.9	1.9	0.1	0.770
Leaf area (cm ²) *	54.6	68.8	1517.6	8.8	0.006
Leaf number	6.5	6.0	1.5	1.9	0.177
Stem diameter (cm)	0.2	0.3	0.0	1.0	0.320
<i>Ranunculus hispidus</i>					
Flower number *	0.2	0.6	1.5	10.9	0.003
Leaf number *	6.3	3.9	42.0	11.1	0.003
Tiller number *	0.6	0.3	1.0	5.8	0.024
<i>Solidago flexicaulis</i>					
Height (cm) *	19.0	27.1	503.2	9.4	0.005
Leaf area (cm ²) *	31.0	48.1	2207.9	16.1	0.001
Leaf number *	5.4	7.7	40.6	12.3	0.002
Stem diameter (cm)	0.2	0.2	0.0	0.1	0.828

exhibited genetically based differences for 15 of the 22 traits we analyzed (some traits, such as tiller and flower number in the greenhouse for *R. hispidus*, and seed head number in the field for *S. flexicaulis* developed too rarely to analyze). The purpose of the greenhouse study, with uniform environmental conditions, was to evaluate these potential genetic differences between the two source populations.

Because the field study occurred at different sites and across years, it also introduced the possibility that we would detect plasticity due to environmental variation that can occur across sites and especially across years. Based on the field study, we also detected phenotypic plasticity. The evidence for this was that the number of trait differences declined with time, with no differences observed in 2016, suggesting that both local and non-local plants were adapting plastically to the local environment. We do not believe subsampling impacted this conclusion, as effect sizes were very small in 2016. We found no evidence that either local or non-local plants outperformed one another in any facet of this study.

Research on plasticity dates back to at least 1922 (Turrenson 1922). In the 1980's plasticity became widely recognized

in the theoretical literature as ubiquitous among plant species as a means to adjust traits to the short-term changes in environmental conditions that occur from year to year (as reviewed by Bazzaz and Sultan 1987). The importance of plasticity continues to be of interest as evidenced by discussions in the theoretical literature and has been noted as “one of the most common phenomena characterizing the living world” (Pigliucci 2005). However, phenotypic plasticity has received little attention in the restoration literature (Falk et al. 2001), even though the implications of plasticity for transplant success and seed collection zones for restoration are potentially important for at least three reasons.

The literature on local ecotypes reflects much concern among restorationists that non-local genotypes will overperform or under-perform compared to local ecotypes, resulting in failed restoration projects. For example, two concerns are that non-local stock will compromise the genetic integrity of plants already present on the restoration site via genetic swamping, or will not succeed due to outbreeding depression (Millar and Libby 1989, Hufford and Mazer 2003, Selbo and Snow 2005). However, if a significant number of plant species have plastic responses to

Table 4. Means (2015) and two-way ANOVA results (block results not shown) for traits measured for six perennial herbaceous understory species transplanted to three field sites in Ames, Iowa. +F_{1,21} for *Asarum canadense*. Asterisks indicate differences in traits between local and non-local plants.

Species/trait	Local mean	Non-local mean	MSE population	+F _{1,26}	p-value
<i>Asarum canadense</i>					
Flower/fruit number	0.8	0.9	0.0	0.1	0.749
Leaf area (cm ²)	37.8	50.5	1155.1	2.7	0.117
Leaf number	2.6	3.1	1.6	2.3	0.147
Stem diameter (cm) *	0.3	0.4	0.0	8.9	0.007
<i>Hydrophyllum virginianum</i>					
Flower number *	11.0	0.5	825.1	7.1	0.013
Leaf area (cm ²)	99.5	103.3	103.4	0.0	0.846
Leaf number	4.3	3.5	5.8	2.3	0.146
<i>Mertensia virginica</i>					
Flower number *	77.3	34.3	13874.7	60.4	< 0.001
Height (cm)	8.5	7.5	7.6	3.8	0.062
Leaf area (cm ²) *	8.3	14.4	274.6	58.6	< 0.001
Leaf number *	27.9	125.2	1205.5	43.8	< 0.001
Stem diameter (cm) *	0.4	0.54	0.2	109.6	< 0.001
<i>Polygonum virginianum</i>					
Height (cm)	28.1	29.1	6.3	0.1	0.770
Leaf area (cm ²)	45.0	59.3	1542.3	2.5	0.123
Leaf number *	6.7	5.3	14.0	5.1	0.033
Stem diameter (cm)	0.3	0.3	0.0	0.0	0.978
<i>Ranunculus hispidus</i>					
Flower number	0.3	0.5	0.3	0.9	0.360
Leaf number	7.0	7.0	0.0	0.0	0.973
Tiller number	0.8	0.7	0.1	0.0	0.844
<i>Solidago flexicaulis</i>					
Height (cm)	26.2	28.3	23.0	0.3	0.577
Leaf area (cm ²)	39.8	38.6	22.9	0.1	0.723
Leaf number	6.6	6.5	0.3	0.0	0.861
Stem diameter (cm)	0.2	0.2	0.0	0.7	0.411

environmental variation in the field, the genetic variation revealed by common garden studies and other measures may not translate well into functional or fitness differences in the field over time (Miner et al. 2005).

Therefore, a second implication of plasticity is that if response to environmental variation is plastic, rather than swamping local ecotypes or underperforming, plants may be able to simply express the appropriate phenotype required by any differences in environment between the source and the restoration site. This would then lessen the focus on strict adherence to locally-sourced seeds or transplants.

Related to this, a third implication is that if plastic responses are prevalent within a species (rather than inflexible genotypes), the potential seed transfer zone may be larger than it would be for species that have genetically subdivided populations. In other words, plastic responses of traits to variations in climate, soil, moisture, temperature, biotic and other factors suggest that the size of appropriate collection zones may be larger than the size often indicated based only on the concept of local ecotypes resulting from local genetic adaptation. However, this is not to say that

important genetic differences never exist. For example, one long-term study found that morphological differences could still be detected between local and non-local populations of *Andropogon gerardii* (big bluestem) even 22 years after the non-local population was planted on a restoration site (Gustafson et al. 2001).

Our study suggests that at this scale (source populations approximately 250 kilometers apart) phenotypic plasticity allows both local and non-local genotypes to adapt to the local environment. If this is more generally the case, it would allow sourcing zones to be relaxed somewhat, giving practitioners greater flexibility in sourcing plant material.

However, it is important to acknowledge that actual collection zones for most forest herbaceous and other species are unknown (e.g., Millar and Libby 1989, McKay et al. 2005, Saari and Glisson 2012, Herman et al. 2014). A fruitful next step would be for restorationists and land managers to ensure that long-term monitoring is included in restoration protocols. Monitoring has been noted as the most feasible way to determine appropriate collection zones (Millar and Libby 1989, McKay et al. 2005, Saari and Gleason 2005). In addition, our results from the third

Table 5. Means (2016) and two-way ANOVA results (block results not shown) for traits measured for four perennial herbaceous understory species transplanted to three forest field sites, Ames, Iowa. Asterisks indicate differences in traits between local and nonlocal plants.

Species/trait	Local mean	Non-local mean	MSE population	F-ratio	p-value
<i>Asarum canadense</i>					
Fruit number	1.2	0.9	0.6	0.7	0.408
Leaf area (cm ²)	138.7	125.6	620.0	0.1	0.714
Leaf number	5.0	5.6	1.1	0.1	0.718
<i>Hydrophyllum virginianum</i>					
Flower number	29.4	26.9	28.3	0.1	0.778
Leaf number	7.3	7.6	0.4	0.1	0.812
<i>Mertensia virginica</i>					
Flower number	40.0	29.6	478.8	2.3	0.149
Leaf number	17.6	20.7	42.5	0.9	0.366
<i>Ranunculus hispidus</i>					
Flower number	1.2	1.3	0.1	0.1	0.746
Leaf number	8.2	8.2	0.8	0.1	0.799
Tiller number	3.6	2.9	0.6	0.1	0.769

year of sampling, where we measured a subset of traits and species, suggest that long-term monitoring may not need to be as extensive as early monitoring.

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Emily Altrichter, HDR Consultants Inc., Omaha, NE.

Catherine M. Mabry, (corresponding author), Department of Natural Resource Ecology and Management, 339 Science Hall II, 2310 Pammel Drive, Iowa State University, Ames, IA 50011.

Janette R. Thompson, Department of Natural Resource Ecology and Management, Iowa State University, Ames, IA.

Randall K. Kolka, U.S. Forest Service Northern Research Station, Grand Rapids, MN.
