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Capturing Genetic Variation during Ecological Restorations: An Example from Kankakee Sands in Indiana

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ABSTRACT

Genetic variation in populations, both natural and restored, is usually considered crucial for response to short term environmental stresses and for long term evolutionary change. To have the best chance of successful long-term survival, restored populations should reflect the extant variation found in remnants, but restored sites may suffer from genetic bottlenecks as a result of founder effects. Kankakee Sands is a large-scale restoration being conducted by The Nature Conservancy (TNC) in northwestern Indiana. Our goal was to test for loss of genetic variation in restored plant populations by comparing them with TNC's seed source nursery and with local remnant populations that were the source of nursery seed and of the first few restored sites. Allozyme analysis of *Baptisia leucantha, Asclepias incarnata, Coreopsis tripteris,* and *Zizia aurea* showed low levels of allozyme diversity within all species and reductions in polymorphism, alleles per locus, and expected heterozygosity between remnants and restorations for all species except *A. incarnata.* Almost all lost alleles were rare; restored populations contained almost 90% of alleles at polymorphic loci that occurred in remnants at frequencies greater than one percent. Allele frequencies for most loci did not differ between remnants and restored sites. Most species showed significant allele frequency differentiation among remnant populations and among restored sites. Our results indicate that seed collection techniques used at Kankakee Sands captured the great majority of allozyme variation present in seed source remnant populations.

Key words: allozyme, ecological restoration, genetic variation, prairie

INTRODUCTION

The goal of ecological restoration is to re-establish historical ecosystem function in degraded or disturbed sites (Society for Ecological Restoration International Science & Policy Working Group 2004). Fundamental to this effort is the recreation of plant communities with the attributes of historical vegetation comprised of resilient, self-sustaining populations. Care is usually taken to include only species known to have been present prior to disturbance (Millar & Libby 1989) and to re-establish historical patterns of hydrology and other ecosystem processes (Society for Ecological Restoration International Science & Policy Working Group 2004). Less attention has been paid to genetics of plant populations in restorations, beyond the now widely appreciated notion that locally collected seed stock may contain locally adapted genotypes (variously referred to as ecotype collection, eco-sourcing, or provenance-based sourcing) (Gustafuson et al. 2004b; Millar & Libby 1989; Handel et al. 1994; Montalvo et al. 1997; Hufford & Mazer 2003). Not much is known about how well patterns of genetic variation in restorations reflect patterns in natural seed source populations (Montalvo et al. 1997; Gustafson et al. 2004a). Genetic variation is hypothesized to be needed for long-term evolutionary change and short-term environmental adaptation (Ellstrand & Elam 1993; Guerrant & Pavlik 1997; McKay et al. 2005) and, thus, has the potential to influence restoration success (Huenneke 1991; Fenster & Dudash 1994; Knapp & Dyer 1998). Plant genetic diversity can also further the goals of ecological restoration by enhancing arthropod diversity and increasing annual net primary productivity (Crutsinger et al. 2006).

The Nature Conservancy (TNC) is undertaking a large-scale ecological restoration in northwestern Indiana (Figure 1). Populations of prairie plants are the foundation of this

restoration. Due to habitat conversion, prairie vegetation in the state is estimated to have been reduced from more than 2 million acres in presettlement times to fewer than 1,000 acres at present (Bacone 1997). The remaining habitat patches are small, fragmented, and isolated. The Efroymson Restoration at Kankakee Sands is a 20,000 acre project to return marginal farmland to a historical matrix of wetlands, sand prairie and *Quercus velutina* (Black oak) savannah, while linking several high quality remnant natural areas. The goal, largely focused on wildlife such as grassland birds, migrating shorebirds and amphibians, is to mitigate effects of fragmentation by increasing connectivity and population size (O'Leary & Shuey 2003) (Figure 1). Natural hydrology has been re-established by removing drainage tiles and ditches. To provide a steady source of seeds for sowing into restored sites, an on-site nursery cultivates plants grown from seeds collected in local remnant prairies (O'Leary & Shuey 2003).

We examined levels of allozyme variation in four perennial plant species in the Kankakee Sands nursery and restored sites and compared them to levels in local natural remnants to see if seed collection and planting techniques are capturing representative genetic variation. To have the best chance of successful long-term survival, restored populations should reflect the extant variation found in remnants, assuming these remnants are healthy (Millar & Libby 1989; Fenster & Dudash 1994; Guerrant 1996). If sampling bottlenecks occur, then it is unlikely that seeds collected from remnants would capture all genetic variation present. Restored populations would then be genetically depauperate, and, if they remained small, would possibly be subject to additional negative effects of inbreeding and genetic drift (Lofflin & Kephart 2005).

METHODS

Background on seed collection techniques at Kankakee Sands - Restorationists at Kankakee Sands followed the advice of Millar and Libby (1989) for seed source material: "Collect it yourself, ideally on or near the restoration site." TNC staff hand collected seed from natural populations located within 80 km of the restoration site and established an on-site nursery. Species with broad habitat requirements that would form the core of replanted areas were the initial focus of collection. Plants with seeds that are hard to collect in the wild, and are therefore worth the effort to propagate, were also a priority for the nursery (O'Leary & Shuey 2003).

Collection efforts attempted to maximize the chances of capturing seed with the widest possible range of genetic variation in traits that may affect plant fitness (Guerrant & Pavlik 1997). Remnant sites ranged in size from one acre to more than 50 acres. TNC collected from many plants of all sizes distributed throughout each site and from some sites over several years. Thus, hundreds of seeds were used to start most of the 150 species being cultivated in the nursery at the time of our study.

Using nursery and wild collected seed, TNC staff constructed species mixes and community groupings based on those found in adjacent high-quality natural remnants. TNC began plantings in 1997 and designed them not to mimic species abundance in remnants, but to "set the planting on an ecological trajectory that will, with time, likely produce a facsimile of a prairie" (O'Leary & Shuey 2003). This was one of a series of decisions and compromises made to achieve goals within operational realities.

Due to incomplete records, we could not track seed from a particular seed source population to individual plants growing in the nursery population. Furthermore, seeds collected from different remnants were mixed before being planted in the restoration sites. Therefore, we could only compare population-level genetic variation in the restored sites and in the nursery with that of remnant sites where seed was collected.

Our study species and procedures - Four herbaceous perennials, *Asclepias incarnata* L. (Swamp milkweed), *Baptisia leucantha* Torr. & Gray (White wild indigo), *Coreopsis tripteris* L. (Tall coreopsis) and *Zizia aurea* (L.) W.D.J. Koch (Golden alexanders) were selected for genetic study during 2001 - 2003. All are common plants of Midwestern prairies and savannahs. They are all primarily outcrossing and insect pollinated (Kephart 1981; Haddock & Chaplin 1982; Lindsey 1984; Crawford et al. 1988). We chose these species because they were present in natural remnants, the TNC nursery, and two or more restored sites. Additionally, they had reproducible and easily interpretable allozyme banding patterns.

We sampled from three site types: remnant, nursery, and restoration (Table 1). The 17 remnant sites included Beaver Lake and Conrad Prairies at Kankakee Sands (Figure 1) and other regional remnant prairie sites within an 80 km radius of the restoration. All of the remnants, except Biesecker Prairie and Spinn Prairie State Nature Preserve and its related sites (Table 1), served as source populations for the nursery and restoration. No site contained all four species. Nursery plants were all first generation plants, grown from wild-collected seed. At the time of our sampling, the nursery contained approximately 200 *B. leucantha*, 1,000 *Z. aurea*, 2,000 *C. tripteris,* and 2,000 *A. incarnata* (Alyssa Nyberg 2006, Kankakee Sands Project, The Nature Conservancy, personal communication). Most plants in the restorations were first generation plants

established from seed. Some of the *A. incarnata* populations in the restored sites established naturally before restoration and were augmented with additional plants during the planting phase of the restoration. The other three species (*B. leucantha, C. tripteris, and Z. aurea*) did not have pre-existing populations in the restoration sites.

We collected leaf material from an average of 30 individuals distributed throughout each site and sent it to Butler University via overnight mail. Enzyme extractions used the modified sorghum buffer of Morden, Doebley and Schertz (1987). Starch gel electrophoresis for allozymes followed standard procedures using recipes from Dolan (1994; 1995). We tested 17 enzymes in each species and scored all resolvable loci, which ranged from a low of 8 to a high of 19 loci (Table 2). Data were analyzed using GDA software of Lewis and Zaykin (1999). We calculated descriptive statistics (e.g., polymorphism, number of alleles/locus, heterozygosity) for each population and overall for each site type (remnant, nursery, restored). We compared distributions of alleles at each locus for remnant versus restored sites using contingency tests generated by Systat software (www.systat.com). Chi-square, likelihood ratio Chi-square, and the application of Fisher's exact test to significance levels, where appropriate, all gave the same results. We include only the Chi-square analysis. Genetic structure of remnants and restorations was analyzed using F-coefficients (Weir 1996). Detailed allele frequency data are available from Dolan.

Results

Polymorphism - Of the 53 loci examined across all four species, 40% were polymorphic. Polymorphism in remnants averaged 37% at the site type level and 15% at the population level (Table 3). All four species showed a decrease in polymorphism at the site type and population level between remnants and restorations, but the level of decrease varied considerably among species. The greatest drop was seen in *B. leucantha*, where 50% of the loci were polymorphic across the 10 remnant populations, but only one locus was polymorphic in the nursery and none in restored sites (Tables 3 and 4). Polymorphism in *C. tripteris*, which was limited to three loci in the remnant populations (Tables 3 and 5), dropped to a single locus in the restorations. In contrast, total polymorphism for *A. incarnata* in restorations was only 14% less than in remnants, and population-level polymorphism decreased by only 5.5% in restorations compared to remnants. Excluding *B. leucantha*, average (± SD) decreases at the site-type level (38 ± 27%) were more than twice as great as decreases at the population level (17 ± 10%).

Allelic diversity - For all species combined, 79 alleles were detected in remnant populations, 69 in the nursery, and 66 in restorations (Figure 2). Allele loss per species ranged from 0% for *A. incarnata* to 36% for *B. leucantha*. *Baptisia leucantha* and *C. tripteris* had fewer alleles in the nursery than in remnants and still fewer in restorations (Figure 2). *Zizia aurea* had a reduction in number from the remnants to the nursery with an intermediate number in restored sites. Allelic richness in *A. incarnata* was approximately equal in all site types, with 30 alleles in the nursery and 29 each in the remnants and restorations. For this species, two alleles found in the nursery were not seen in our screenings of the remnants (Table 6). These alleles were present at low frequencies (1%) and may have been missed by our remnant sampling. Likewise, a low

frequency allele was detected in remnants and restorations but not in the nursery for *Z. aurea* (Table 7). *Asclepias incarnata* restoration sites had one allele not found in sampled remnant sites or the nursery.

Tracking just those 47 alleles at polymorphic loci that we detected in remnants, we found that 13 (28%) and 15 (32%) were not present in the nursery or in restorations, respectively. Losses for individual species varied from 5-10% in *A. incarnata* to about 50% in *B. leucantha* (Figure 3). Most alleles that were not captured in restored sites were present in very low frequencies in the remnants. For example, the frequencies of the eight alleles lost for *B. leucantha* averaged 0.01 (Table 4). When considering only alleles at polymorphic loci present in remnants at greater than 1% frequency, the overall capture rate for all species from the remnants to the restorations was 88.9%.

Most cases of allele frequency shifts between remnant sites and restorations were due to loss of rare alleles in restorations (e.g., all the frequency shifts in *B. leucantha*; Table 4), but we also found a few cases of more major frequency shifts. In *A. incarnata*, the *PGI-a* allele more than doubled in frequency from 0.08 in remnants to 0.18 in restored populations, whereas the *SKD-a* allele decreased by about 76% from 0.36 in remnants to 0.09 in restored sites (Table 6). In *C. tripteris*, the *PGI* locus had very similar allele frequencies in remnants and nurseries, but different frequencies in restorations. The *PGI*-a allele was present at around 50% in remnants and nurseries, but was found in a frequency of 0.28 in the restored sites, while the *PGI-b* allele almost doubled in frequency in restorations (Table 5). At that same locus, the *PGI-c* allele, present in remnants at a frequency of 0.13, was absent in restorations. Last, in *Zizea aurea*, the *IDH-c* allele, present at a frequency of 0.09 in the remnants, was absent from the nursery and restorations (Table 7). Only four of the 21 polymorphic loci examined had

significant differences in allele frequencies between remnant and restored sites (Tables 4-7),

Heterozygosity - Expected heterozygosity (H_E) is the best overall measure of genetic diversity because it incorporates both number of alleles and their frequencies. Three of four species showed decreases in H_E from remnants to restored sites (Table 3). For *B. leucantha*, H_E drops to 0, and for *Z. aurea*, H_E drops by more than 50%. In contrast, mean H_E in restored *A. incarnata* populations was about the same as in remnants. Heterozygosity in the nursery was equal to or slightly greater than that in remnants for both *A. incarnata* and *C. tripteris*, which suggests that future restorations, if seeded from nursery-produced seeds, will have levels of genetic variation similar to local native populations. Nursery populations of *B. leucantha* and *Z. aurea*, on the other hand, showed considerably lower heterozygosity levels than did remnants.

Genetic structure - Analysis of genetic structure was hampered by the low number of polymorphic loci, especially for *B. leucantha* and *C. tripteris*. Confidence limits were large for most analyses and impossible to calculate in the rest (Table 8). For example, *B. leucantha* restorations exhibited no structure because all loci were monomorphic. Remnant populations of *B. leucantha*, however, were variable and showed no evidence of inbreeding (*f* not different from 0), but differed significantly in their allele frequencies ($\theta > 0$). Differences among populations were due primarily to the presence of several low-frequency private alleles (i.e., those alleles found only within one population). Analysis of genetic structure in *C. tripteris* was also weakened by low numbers of variable loci, as the *PGI* locus was the only locus with multiple alleles in more than one remnant population. That locus, however, suggested low levels of inbreeding within remnant populations ($f = 0.082$) and some genetic differences among populations ($F = 0.160$; $\theta =$

0.087). The *PGI-a* allele, for example, varied in frequency from 0.27 to 0.73 across remnant populations, while the *PGI-c* allele varied from 0.0 to 0.25. Frequency data for the *PGI* locus in restored *C. tripteris* populations suggested heterozygote excesses (*f* = -0.179) and frequency differences between restorations (θ = 0.133).

The F-coefficients suggested little evidence of inbreeding within remnant populations of *A. incarnata* (*f* not different from 0), but significant allele frequency differences among populations (both *F* and θ significantly greater than 0) (Table 8). The high θ value (θ = 0.285) reflects several large allele shifts among the three populations sampled, especially at the *ADH1*, *IDH*, and *SKD* loci. The *SKD-a* allele, for example, was found in a frequency of 0.05 in the Beaver Lake population, but in a frequency of 0.67 in the Hog Marsh population. Restored *A. incarnata* populations showed significant heterozygote deficiencies as well as significant frequency differences among the four restorations. Last, *Z. aurea* showed no evidence of inbreeding within remnants (*f* not greater than 0), but allele frequencies differed significantly among populations (θ = 0.060). Restored populations showed a similar level of allele frequency differences (θ = 0.054). Overall, despite the low power of the analyses, significant allele frequency differences were detected among remnants in three species and among restorations in both species for which confidence limits could be calculated.

DISCUSSION

To have the best chance of long-term survival, restored populations should reflect the extant genetic variation found in remnant seed source populations (Knapp & Dyer 1998; Jones & Hayes 1999). Because restorations usually begin with relatively small populations, the amount of genetic variation represented in the founding population can be critical (Montalvo et al. 1997; Gustafson et al. 2001). Barring mutation and gene flow, the initial number of alleles present limits selection response and evolutionary potential (Fernandez et al. 2004). However, if thousands of additional acres are restored as planned at Kankakee Sands, genetic and ecological connectivity with natural prairies and among restorations will be enhanced within a few years. When compared to a survey of over 450 species (Hamrick & Godt 1989), the remnant seed source populations in our study were not especially variable. The four species that we monitored had an overall mean expected heterozygosity (± 1 standard deviation) of 0.041 ± 0.032, which is much lower than the value of 0.096 expected for herbaceous perennials (Hamrick & Godt 1989). Low levels of expected heterozygosity have also been observed in three remnant Illinois populations of *Dalea purpurea* (mean H_{exp} = 0.06 based on 9 allozyme loci; Gustafson et al. 2002). It may be that these sites have themselves suffered negative effects of habitat fragmentation. Beyond the direct loss of genotypes caused by habitat destruction (the "genetics of subtraction" of Schaal & Leverich 2004), genetic variation can be lost over time through inbreeding and genetic drift in small and isolated surviving populations (Barrett & Kohn 1991; Ellstrand & Elam 1998). The presence of a high θ value for *A. incarnata* and the presence of numerous private alleles in *B. leucantha* suggest that gene flow among remnants has historically been low. Under such conditions, genetic drift can act more readily to eliminate variation. Because our collection sites are all within 80 km of each other, however, we

do not know whether the low levels of variation we documented are characteristic of each study species as a whole or whether they represent recent losses due to the negative effects of fragmentation and habitat destruction.

Our results show evidence of decreases in genetic variation in restored sites compared to remnant sites for three of the four species. *Baptisia leucantha*, for example, has apparently lost all variation at the allozyme loci tested. Likewise, *C. tripteris* and *Z. aurea* showed some decreases in all measures of variation (polymorphism, number of alleles/locus, and heterozygosity). *Coreopsis tripteris*, for example, lost all variation at two of three polymorphic loci. Only *A. incarnata* exhibited levels of genetic variation in the nursery and restorations that were comparable to or slightly greater than levels in remnant sites. The *A. incarnata* data may be explained by the presence of natural populations in the restored sites prior to sowing. When we tracked only those alleles detected in remnants, however, even *A. incarnata* had slightly lower allelic richness in the nursery and in restorations than in remnants.

These results agree with the pattern of allelic loss predicted if seed collection strategies are causing genetic bottlenecks. In agreement with population genetic theory (Nei et al. 1975), the large majority of alleles lost were at low frequencies of 1% or less in remnant populations. As a consequence, the overall extent of the bottleneck is not great. Almost 90% of alleles detected in remnants in frequencies of 1% or greater are present in the nursery and in the restorations and are in frequencies that are generally comparable to their occurrence in natural remnants. Only four of 21 polymorphic loci had significant allele frequency differences between remnant and restored sites. Use of a different genetic marker such as randomly amplified polymorphic DNA (RAPD) or microsatellite DNA markers may provide more information on fine-scale genetic differentiation.

Gustafson et al. (2002) used both allozyme and RAPD markers on restored and remnant populations of *Dalea purpurea,* and found that the RAPD markers had higher levels of polymorphism than the allozyme markers (11-33% polymorphic allozyme loci per population vs. 79-100% polymorphic RAPD loci). However, the allozyme analysis provided greater resolution than RAPD markers of population-level relationships between remnant and restored sites at a regional geographic scale (e.g. sites located within Illinois). It would be interesting to repeat our study using different genetic markers to see whether allozyme analysis or another genetic marker provided better resolution of genetic differences among populations.

While there is general agreement that the goal of conservation efforts should be to ensure sufficient genetic variation for long-term population persistence (e.g., Jones 2003, McKay et al. 2005), the importance of rare alleles in the restoration process is debated. Some argue it is not necessary to capture all the genetic variation in species: rare alleles, such as alleles present at frequencies less than 1%, may in fact be deleterious, may not be of the same evolutionary significance as more common alleles, and are likely to be lost in a few generations (Brown & Briggs, 1991; Holsinger & Gottlieb 1991).

At Kankakee Sands, seeds for restorations were collected from multiple local source populations. Smulders et al. (2000) and Gustafson et al. (2004a) found that using seed from more than one source population resulted in restorations with more genetic variation. Knapp and Dyer (1998) proposed that seed mixes collected from different populations in a region could maximize the amount of genetic variation available and increase the chance that the new population could adapt, over time, to many possible environments. Guerrant et al. (2004) recommended collection of seed from up to 50

populations, if practical, for restorations of rare plant populations. On-going collections at Kankakee Sands have been made from 125 different remnant sites, averaging five sites per species (Alyssa Nyberg 2006, Kankakee Sands Project, The Nature Conservancy, personal communication).

Despite the low statistical power of our genetic structure analysis, three of four species showed significant allele frequency differences among remnant populations. This finding supports the benefit of collecting seed from multiple remnants for the project; they are not all equal genetically. Possibly due to the vagaries of seed collection from year to year, restored populations also showed allele frequency differences from one another. This suggests that at a landscape scale, Kankakee Sands restorations are mirroring natural genetic patterns.

Few other studies have investigated genetic variation in community-level multi-species restorations created using locally-collected seed stock. In a similar study to ours, first generation reintroduced plants of *Cirsium dissectum* and *Succisa pratensis* contained fewer amplified polymorphic (AFLP) bands than source populations in Dutch grasslands (Smulders et al. 2000). The difference was attributed to founder effects due to use of a limited number of seeds to establish restorations. Van Treuren et al. (2005) used AFLPs to show that populations of native perennial rye grass (*Lolium perenne*) and white clover (*Trifolium repens*) in recently protected nature preserves had the same range of variation as populations from old Dutch grasslands under grazing regimes. Only a small number of low-frequency alleles found in old Dutch grasslands were absent from the nature preserves. Gustafson et al. (2002) used allozymes and RAPD markers and found that genetic diversity in *Dalea purpurea* was higher in restored prairies compared to remnant prairies. The restored populations had been established for 15 – 40 yrs, and the higher

genetic diversity was attributed to collecting seed from multiple regional source populations. Finally, Wells et al. (2003) used AFLPs to show that seed of the grass *Triodia bitextura* used in Australian restorations collected within 30 km of the restoration area were genetically representative of native populations.

To the extent that allozyme diversity reflects total genome diversity, our results indicate that seed collection and planting techniques used at Kankakee Sands are generating stock for restorations that reflects the qualitative and quantitative variation found in remnant seed source sites. Continued restoration from the nursery stock, coupled with additional collections from local populations, should increase overall levels of genetic variation for all restored species and should generate a set of restored populations that closely mimics the levels of genetic variation and population structure currently found in northwest Indiana remnants.

Restoration is on-going at Kankakee Sands with a goal of restoring 500 acres a year. These efforts will increase connectivity and the opportunity for gene flow within and between restored and remnant plant populations, further mitigating habitat fragmentation and restoring ecosystem function (Handel et al. 1994). McKay et al. (2005) call for using restorations as research sites to study ecological genetics. Although lacking the detailed provenance records called for by Gustafson et al. (2001), our baseline data provide the opportunity to track genetic changes at Kankakee Sands. In addition, comparative demographic studies of performance and fitness of plants in Kankakee Sands restorations and seed-source remnants are needed to continue to evaluate the success of the plantings. Guerrant and Pavlik (1997) suggest that a minimum of ten years is needed to allow physical and biological factors to be expressed in restorations. This would allow adequate time to evaluate responses at the population level to gene flow

and at the community level to temporal environmental variation, including environmental extremes (Knapp & Dyer 1998).

Implications for Practice

- This case study suggests that restoration techniques used at Kankakee Sands are capturing most of the genetic variation present in local seed source populations for four prairie plants.

- These techniques include an on-site nursery for amplification of locally collected seed and collection from multiple seed source populations.

- Landscape-scale restorations such as Kankakee Sands can serve as experimental sites for investigations in ecological and genetic restoration.

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Table 1. Sites sampled for each species. Remnants are natural prairie sites. Three restoration sites are named according to the year in which they were planted (1997, 1998, 1999), and two sites are designated by their TNC unit name (D and K).

Collection site	Location Co., State	*Asclepias incarnata*	*Baptisia leucantha*	*Coreopsis tripteris*	*Zizia aurea*
Remnants					
Beaver Lake Prairie Nature Preserve	Newton Co., IN N 41°3' W 87°24'	X			
Biesecker Prairie	Lake Co., IN N 41°25' W 87°28'		X	X	X
Conrad Savanna Nature Preserve	Newton Co., IN N 41°05' W 87°28'		X		
German Methodist Cemetery Prairie	Lake Co., IN N N 41°19'' W 87°28'			X	X
IL 52	Iroquois Co., IL N 40°47.5' W 87°34'			X	
Hog Marsh, Grand Kankakee Marsh	Newton Co., IN N 41°12' W 87°15'	X			

IN 24	Newton Co., IN N 40°46’ W 87°30’			X	X
IN 24/IL 52	Iroquois Co., IL N 40°46’ W 87°33’			X	X
Iroquois County Conservation Area	Iroquois Co., IL N 40°59’ W 87°33’		X	X	
Iroquois check-in	Iroquois Co., IL N 40°59’ W 87°35’		X		
Jasper-Pulaski	Jasper Co., IN N 41°08’ W 86°55’		X		
Monon Railroad (near Spinn Pr.)	White Co., IN N 40°51’ W 86°52’		X		
North Judson	Starke Co., IN N 41°14’ W 86°47’		X		
Spinn Prairie State Nature Preserve	White Co., IN N 40°47’ W 86°52’		X		
Spinn east marsh	White Co., IN	X			

	N 40°47’ W 86°51.5’				
Spinn south end	White Co., IN N 40°46’ W 86°52’		X		
Stoutsberg Savanna Nature Preserve	Jasper Co., IN N 41°10’ W 87°05’		X	X	X
Nurseries					
TNC nursery	Newton Co., IN N 41°02’ W87°23’	X	X	X	X
TNC field house	Newton Co., IN N 41°02’ W87°26’			X	
Restorations					
1997	Newton Co., IN N 41°05’ W87°25’	X	X	X	X
1998	Newton Co., IN N 41°03.5’ W87°24’		X	X	X
1999	Newton Co., IN N 41°03’ W87°24’	X			

Area D	Newton Co., IN N 41°03.5’ W87°24.5’	X
Area K	Newton Co., IN N 41°02’ W87°26’	X

Table 2. Enzyme systems and number of loci resolved for each species.

Enzyme system	*Asclepias incarnata*	*Baptisia leucantha*	*Coreopsis tripteris*	*Zizia aurea*
Acid phosphatase	1		1	
Aconitase	2		1	
Alcohol dehydrogenase	2	1	1	2
Esterase		3	1	
Glucose-6-phosphate dehydrogenase	1			
Glutamate dehydrogenase				1
Glyceraldehyde-3-phosphate dehydrogenase	1	1		
Isocitrate dehydrogenase	1	1		1
Malate dehydrogenase	1	4	1	1
Malic enzyme	1			
Menadione reductase	2	2	1	
Peroxidase	1		1	1
Phosphoglucomutase				1
Phosphoglucose isomerase	1		1	
6-phosphogluconate dehydrogenase	1	1	1	
Shikimate dehydrogenase	2	1	1	
Triosephosphate isomerase	2		2	1
Total number of loci	19	14	12	8

Table 3. Summary of descriptive genetic statistics.

Species/Site type	No. Sites	N	P_{Tot}	P_{Pop}	A_{Tot}	A_{Pop}	$A_{Tot/Poly}$	$A_{Pop/Poly}$	H_E
A. incarnata									
Remnant	3	26.0	36.8	23.8	1.5	1.3	2.4	2.4	0.071
Nursery	1	72.0	31.6	31.6	1.6	1.6	2.8	2.8	0.087
Restoration	4	28.9	31.6	22.5	1.5	1.3	2.7	2.3	0.077
B. leucantha									
Remnant	10	28.2	50.0	9.3	1.6	1.1	2.1	2.1	0.011
Nursery	1	25.0	7.1	7.1	1.1	1.1	2.0	2.0	0.006
Restoration	2	30.0	0.0	0.0	1.0	1.0	-	-	0.000
C. tripteris									
Remnant	7	26.1	25.0	10.7	1.3	1.2	2.3	2.6	0.047
Nursery	2	35.0	16.7	12.5	1.3	1.2	2.5	2.5	0.050
Restoration	2	15.0	8.3	8.3	1.1	1.1	2.0	2.0	0.032
Z. aurea									
Remnant	5	26.4	37.5	16.4	1.5	1.2	2.3	2.1	0.039
Nursery	1	23.0	12.5	12.5	1.1	1.1	2.0	2.0	0.020
Restoration	2	18.0	25.0	12.5	1.3	1.1	2.0	2.0	0.019

N = Mean number of plants sampled per population

P_{Tot} = Percentage of polymorphic loci at the site type level

P_{Pop} = Mean percentage of polymorphic loci at the population level

A_{Tot} and A_{Pop} = Mean number of alleles per locus at the site type and population levels

$A_{Tot/Poly}$ and $A_{Pop/Poly}$ = Mean number of alleles per polymorphic locus at the site type and population levels

H_E = Expected heterozygosity averaged over loci and populations within site type

Table 4. Allele frequencies for the seven polymorphic loci in *Baptisia leucantha* pooled by site type. The X^2 values test for differences between pooled remnant and restoration frequencies.

Locus	Allele	Remnant	Nursery	Restoration	X^2
ADH	a	0.992	1.000	1.000	0.86
	b	0.008	0.000	0.000	
EST1	a	0.004	0.000	0.000	0.40
	b	0.996	1.000	1.000	
EST2	a	0.990	1.000	1.000	1.30
	b	0.010	0.000	0.000	
EST3	a	0.998	1.000	1.000	0.22
	b	0.002	0.000	0.000	
IDH	a	0.003	0.040	0.000	2.40
	b	0.980	0.960	1.000	
	c	0.017	0.000	0.000	
6PGD	a	0.970	1.000	1.000	3.74
	b	0.030	0.000	0.000	

MNR	a	0.992	1.000	1.000	0.86
	b	0.008	0.000	0.000	

*** = p<0.001; * = p<0.05

Table 5. Allele frequencies for the three polymorphic loci in *Coreopsis tripteris* pooled by site type. The X^2 values test for differences between pooled remnant and restoration frequencies.

Locus	Allele	Remnant	Nursery	Restoration	X^2	
PGI	a	0.506	0.557	0.275	30.35	***
	b	0.367	0.343	0.725		
	c	0.126	0.100	0.000		
TPI1	a	0.003	0.000	0.000	0.17	
	b	0.997	1.000	1.000		
TPI2	a	0.997	0.986	1.000	0.17	
	b	0.003	0.014	0.000		

*** = $p<0.001$; * = $p<0.05$

Table 6. Allele frequencies of the eight polymorphic loci in *Asclepias incarnata* pooled by site type. The X^2 values test for differences between pooled remnant and restoration frequencies.

Locus	Allele	Remnant	Nursery	Restoration	X^2
ADH1	a	0.733	0.830	0.710	0.26
	b	0.267	0.170	0.290	
G3PDH	a	0.990	1.000	1.000	2.99
	b	0.010	0.000	0.000	
IDH	a	0.418	0.530	0.503	3.57
	b	0.552	0.420	0.495	
	c	0.030	0.050	0.003	
MDH	a	1.000	1.000	0.998	0.67
	b	0.000	0.000	0.003	
PGI	a	0.083	0.140	0.178	7.56
	b	0.037	0.020	0.010	
	c	0.850	0.780	0.758	
	d	0.000	0.010	0.020	
	e	0.030	0.050	0.035	

PGM	a	0.980	0.980	0.970	0.45	
	b	0.020	0.020	0.030		
SKD	a	0.360	0.200	0.088	44.06	***
	b	0.640	0.800	0.913		
TPI	a	0.000	0.010	0.000	2.99	
	b	0.990	0.960	1.000		
	c	0.010	0.030	0.000		

*** = p<0.001; * = p<0.05

Table 7. Allele frequencies for the three polymorphic loci in *Zizia aurea* pooled by site type. The X^2 values test for differences between pooled remnant and restoration frequencies.

Locus	Allele	Remnant	Nursery	Restoration	X^2	
ADH2	a	0.978	1.000	0.962	0.53	
	b	0.022	0.000	0.038		
IDH	a	0.010	0.000	0.000	8.91	*
	b	0.903	1.000	1.000		
	c	0.087	0.000	0.000		
TPI2	a	0.865	0.910	0.957	11.35	***
	b	0.135	0.090	0.043		

*** = $p<0.001$; * = $p<0.05$

Table 8. Mean F-coefficients across all polymorphic loci for remnants and restorations, calculated according to the methods of Weir (1996), as implemented in GDA 1.0d15 (Lewis & Zaykin 1999). The 95% confidence limits (in parentheses) were determined through bootstrapping over loci. Positive *f* and *F* values suggest deficiencies of heterozygotes relative to Hardy-Weinberg expectations within populations and within site types, respectively. Values of θ measure degree of allele frequency differences among populations.

Species/Site type	*f*	*F*	θ
A. incarnata			
Remnant	0.212	0.436	0.285
	(-0.100-0.637)	(0.070-0.756)	(0.064-0.671)
Restored	0.269	0.315	0.063
	(0.081-0.530)	(0.104-0.589)	(0.019-0.127)
B. leucantha			
Remnant	0.085	0.150	0.071
	(-0.125-0.545)	(-0.017-0.571)	(0.025-0.107)
Restored[1]	----	----	----
C. tripteris			
Remnant	0.080	0.160	0.087
	(0.003-0.082)	(-0.001-0.163)	(-0.003-0.088)

Restored[2]	-0.179	-0.022	0.133
Z. aurea			
Remnant	0.221	0.267	0.060
	(-0.110-0.375)	(0.006-0.443)	(0.010-0.109)
Restored	-0.063	-0.005	0.054
	-0.067-(-0.055)	(-0.021-0.027)	(0.043-0.077)

[1]Could not be calculated, because populations were not variable.

[2]95% confidence limits could not be calculated, because only a single locus was variable.

Figure Legends

Figure 1. Map of The Nature Conservancy's Kankakee Sands restoration site in Newton County, Indiana.

Figure 2. Total number of alleles detected in remnant prairies and in the Kankakee Sands nursery and restoration sites.

Figure 3. Summary of allelic loss at polymorphic loci during the restoration process. Data are number of alleles detected in remnants, and of those, the numbers detected in nursery and restoration sites at Kankakee Sands.