

**Final report, National Fish and Wildlife Foundation Grant  
Midewin Tallgrass Prairie Restoration Fund Proposal 11439**

*Evaluating Provenance Limits in Prairie Sedges:  
Development of Microsatellite Markers in *Carex scoparia**

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**OVERVIEW OF PROJECT GOALS**

Sound ecological restoration depends on sampling appropriately at two scales: from the local flora and from the local gene pool. Even if species selection for a prairie restoration is appropriate, genotypes from outside of the region pose potential ecological risks through (1) introduction of maladaptive nonlocal alleles or (2) invasiveness of nonlocal genotypes. Consequently, restorationists frequently employ a rule-of-thumb approach to collecting plants or seeds, limiting the geographic range from which they collect. However, empirical tests of these rules of thumb are sorely lacking. Two types of data are needed to the question of “how far is too far” in collecting seed for ecological restorations: population genetic data to characterize patterns of gene flow, and quantitative ecological data to characterize how plants respond to relocation.

The goals of this project were to **(1)** develop population genetic markers needed to test the Midewin Land and Resource Management Plan’s (LRMP) guidelines regarding collecting provenance limits for sedges (*Carex*: Cyperaceae); **(2)** increase integration between restoration ecology and molecular genetics by providing postdoctoral training in molecular conservation genetics for a restoration ecologist; and **(3)** assess genetic divergence between study populations of *C. scoparia*, and compare with another wind-pollinated species group (viz., oaks). All goals were achieved in the course of this work, as summarized below.

**PROJECT ACCOMPLISHMENTS**

**Activity 1: Develop microsatellite markers for use in *C. scoparia* and relatives.** We have developed 40 microsatellite markers, of which 16 show excellent variability among species. This exceeds our expected outcome of 4–10 microsatellites and provides raw materials (i.e., approximately 20 additional primer pairs) for future studies in *Carex scoparia* and related species.

**Activity 2: Provide postdoctoral training in conservation genetics to a wetland restorationist.** We hosted Karin Kettenring (currently a postdoctoral fellow at the Smithsonian Institute) for 2 visits, during which time she received training in microsatellite development and interpretation. Our collaboration is ongoing, and she will be the first author on the published microsatellite primers.

**Activity 3: Assess population genetic structure of *Carex scoparia*, and compare with population genetic structure of oaks from previous and ongoing work in our lab.** We have screened nine of the primer pairs we developed on a sample of 43 *C. scoparia* individuals from throughout much of the range of the species that were collected by collaborator P. Rothrock (Taylor University) as part of a separate project. We analyzed the data using a Bayesian population assignment method as implemented in the software package STRUCTURE (J. Pritchard, U. of Chicago). We used this method to ask the question of how many populations it takes to best define the genetic structure of the species. While this is not an ideal way of investigating genetic structure when the correct genetic model may be isolation-by-distance, we did this to compare our findings with a study that we have in press of population genetic

structure in three oak species (*Quercus velutina*, *Q. ellipsoidalis*, and *Q. coccinea*; funded by American Philosophical Society, not by the current grant). The difference between the two studies is striking. Using nine primer pairs in *Carex scoparia*, we are able to distinguish at least four clear population groups with some individuals representing hybrids between those population groups (Figure 1). These population groups do not sort out neatly by geography (Table 1). Instead, they appear to segregate better by chromosome number, suggesting that chromosome number may be an important determinant of population genetic structure within this species, which ranges from  $2n = 58$  to  $2n = 70$ .



**Figure 1. Bayesian analysis of population genetic structure in *Carex scoparia* based on nine microsatellites.** Analysis was conducted in STRUCTURE 2.1 ([pritch.bsd.uchicago.edu/structure.html](http://pritch.bsd.uchicago.edu/structure.html)) using the admixture model, allele frequencies correlated between populations, excluding prior information on geographic location of samples and assuming  $K = 4$  populations based on trial analyses of  $K = 1$  to  $K = 8$  populations. Bars represent individuals' genotypes, and the Y-axis represents the cumulative percent of an individual's genotype inferred to have derived from any of the four ancestral populations. 36 of 43 individuals analyzed have >95% of their ancestry from a single source population, suggesting that the clusters recovered in this analysis are genetically distinct from one another.

Population cluster	Mean $2n$ $\pm$ S.E.M.	IL (1)	IN (4)	ME (6)	MO (2)	NC (7)	NH (1)	NY (1)	OK (1)	PA (1)	TN (3)	VA (9)	VT (1)	WI (1)	WV (5)
Red (1)	$67.8 \pm 0.8028$	X			X	X				X		X			X
Green (2)	$65.6 \pm 0.3786$			X		X						X	X		X
Blue (3)	$63.9 \pm 0.7896$		X		X			X	X		X	X		X	
Yellow (4)	$61 \pm 0.9623$					X	X				X	X			

**Table 1. Summary of chromosome numbers and geographic source of individuals screened.** Population clusters refer to the clusters shown in Figure 1 (numbered from left to right). Individuals were assigned to a population if they had  $\geq 90\%$  inferred ancestry from any single population. By this criterion, five of 43 individuals were excluded from analysis (cf. Figure 1). Chromosome numbers are reported as the mean  $\pm$  standard error of the mean. State abbreviations are followed by the number of individuals sampled from each state in parentheses. While there is clearly some geographic correlation to the genetic structure—e.g., all six Maine samples fall in cluster 2—the lack of geographic structure is striking, with West Virginia, Missouri, and Tennessee samples falling in two clusters each, North Carolina samples falling in three clusters, and Virginia samples falling in all four clusters. In distinction, the distribution of chromosome numbers strongly suggests that populations are sampling non-randomly from the chromosome numbers observed. This finding is supported by previous analyses we have conducted using AFLP data and suggests that chromosome number may in fact be more important in seed provenance decisions in sedges than geographic distribution.

For comparison, we evaluated population genetic structure in *Quercus coccinea* from three widely separated populations (southern IL, southern OH, and eastern MO) using AFLP data and were unable to detect any population genetic structure; individuals could not be reliably assigned to population despite the wide distance between populations (data not shown here, in press in *Systematic Botany*). We also evaluated three Chicago region sites that contain both *Quercus ellipsoidalis* and *Q. velutina* using nine microsatellites and found extensive evidence of hybridization between the two. This lack of differentiation between populations within species and limited differentiation between species contrasts starkly with the population genetic structure we find within *Carex scoparia*.

## IMPLICATIONS FOR PROVENANCE IN RESTORING PRAIRIE SEDGES

The Midewin Land and Resource Management Plan's (LRMP) guidelines regarding collecting provenance limits provide the following standard on seed collecting: "Only seed collected from appropriate and local seed sources for restoration of native vegetation shall be used either directly on lands selected for restoration, or to establish seed production beds and fields on-site" (Chapter 4.2.1.3). The guidelines that follow define a nested hierarchy of seed-collecting regions: (1) existing remnants on Midewin and adjacent

Prairie Parklands; (2) northern Grand Prairie Natural Division and southern NE Morainal Division of IL; (3) elsewhere in the Grand Prairie and NE Morainal Divisions in IL and IN; and (4) sources beyond these limits in the NW quarter of IN, the northern two-thirds of IL, and the SE quarter of WI. For this last limit, the guidelines specify that seed be limited to wind pollinated species such as sedges, rushes, grasses, and oaks.

Our findings to date suggest that life history needs to be taken into account in setting provenance guidelines. In particular, we find no reason based on the neutral genetic variance assessed in the current study to limit sedge or oak collections based on geography. Across an exceedingly wide geographic range, oaks and sedges show limited genetic structure. However, the chromosomal variance in sedges may bear strongly on neutral population genetic structure. In our current work (described below), we investigate this at a local level using within- and between-population sampling of both genetic and chromosomal variance. Moreover, genes under selection may show much stronger population genetic structure than the (presumably) neutral loci sampled in this study. Future work (also described below) will address this directly.

### **FUTURE WORK BASED ON THIS PROJECT**

We have procured funding from Chicago Wilderness to investigate population genetic and chromosomal diversity in *Carex scoparia* throughout the Chicago region, and we are currently using the microsatellites developed in this project to survey more than 20 populations for both genetic and chromosomal variation in the coming year. This is the next step in our understanding of the effects of provenance decisions in restoring prairie and wetland sedges, and it rests solidly on the results of the NFWF funding we received through the Midewin Tallgrass Prairie Restoration Fund. Our understanding of population genetic structure based on the neutral population genetic markers we have developed in this study also builds a foundation for the next steps of this project, which will entail crossing experiments and reciprocal transplant experiments between populations with differing chromosome numbers to assess the effects of chromosome number, habitat differences, and geographic distance on local adaptation. This, in the end, will allow us to solidly advise the restoration community on whether and to what extent provenance decisions in this sedge species and its relatives should rest on geography, chromosome number, and ecology.