

An investigation of hybridization between the  
threatened *Solidago sciaphila* (cliff goldenrod)  
and the common *Solidago hispida* (hairy goldenrod)

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## Introduction

The role of hybridization in plant evolution continues to be debated (e.g., Arnold 1997) and in conservation biology it is a controversial issue. Rules pertaining to the protection of hybrids under the Endangered Species Act have recently been revised, allowing hybrids protection under several circumstances (reviewed in Soltis and Gitzendanner 1999). However, hybridization with a congener may cause the rare species to be genetically 'assimilated' into the widespread species (reviewed in Levin et al. 1996; Rhymer and Simberloff 1996; Soltis and Gitzendanner 1999). In some cases the introduction of 'new' genetic material could bolster the rare species' genetic diversity and may increase its chance of survival.

*Solidago sciaphila* (cliff goldenrod, Asteraceae) is restricted to the Driftless Area of Iowa, Minnesota, Wisconsin, and northwest Illinois. It is listed as threatened in Illinois (Herkert and Ebinger 2002) and of special concern in Wisconsin (WSH 2003). *Solidago sciaphila* grows on cliff faces and exposed dolomite and sandstone. In Illinois it is known only from Carroll, Jo Daviess, La Salle, and Ogle counties. *Solidago sciaphila* is typically glabrous throughout with the exception of some hairs on the inflorescence and short, stiff hairs on the fruits (achenes) (Salamun 1963).

Often found growing on the ledges above *S. sciaphila*, *S. hispida* (hairy goldenrod) is more widespread and occurs throughout the eastern United States (USDA-NRCS 2003). This species is found in less open areas, near forest edges in areas adjacent to sites where *S. sciaphila* occurs. *Solidago hispida* can be distinguished from *S. sciaphila* with its overall hairiness, and glabrous achenes (Salamun 1963).

In northwestern Illinois, both *S. sciaphila* and *S. hispida* occur along the bluffs adjacent to the Mississippi River and in the Apple River Canyon. *Solidago sciaphila* is, in general,

confined to this area of the Wisconsin Driftless Division bluffs. *Solidago hispida*, however, is found on rock outcrops throughout the Wisconsin Driftless Division, even in the interior far away from the major river bluffs.

We have observed individuals exhibiting variation in the morphological characters used to distinguish these two species; some individuals appear as morphological intermediates. We hypothesize that the two species could be hybridizing, a phenomenon also suggested by Salamun (1963) for sympatric populations in Wisconsin.

The objectives of this study were to (1) sample individuals from several Illinois populations, including what appear to be "true" *S. sciaphila* and *S. hispida*, and populations that contain morphological variants, and (2) develop genetic markers that clearly distinguish *S. sciaphila* and *S. hispida*. In future work, we plan to use these markers to determine if hybridization is indeed occurring between these two species in Illinois and how this might ultimately effect conservation efforts for *S. sciaphila*.

## **Materials and Methods**

### *Population sampling*

Thirty individuals were sampled from each population. Two leaves were removed per individual and placed in a paper coin envelope to dry. Five populations were sampled on 22-23 October 2001 and four populations on 17-18 October 2002 (Table 1). Voucher specimens consisting of an inflorescence and several leaves were collected and will be deposited in the Illinois Natural History Survey's herbarium (ILLS).

## *Molecular Markers*

We decided to pursue DNA-based markers because they are more variable and are better able to distinguish differences between species. Random Amplified Polymorphic DNA (RAPD) marker protocols generally followed those of Koontz et al. (2001), but are briefly discussed here. A representative sample from four of the populations collected in 2002 were used (Apple Canyon, Galena Territories West, Indian Head, and Townsend Rd) for the RAPD surveys. To determine if RAPD markers could be used to distinguish the two species only individuals that we felt were "true" *S. sciaphila* or *S. hispida* were used for these initial RAPD surveys. DNA was extracted from 10 mg of dried leaf tissue using the Wizard DNA extraction Kit (Promega), following the manufacturers instructions; however, the ground leaf material in the nuclei lysis buffer was incubated at 65° C for at least 1 hr. DNAs were quantified with a SmartSpec 3000 spectrophotometer (Bio-Rad) and diluted to a standard 10 ng/ $\mu$ l with TE pH 8.0. Primers from Operon's (Qiagen) A and B RAPD kits were surveyed using a subset of the *Solidago* samples collected. RAPD reactions consisted of a 25  $\mu$ l reaction containing 17  $\mu$ l of sterile distilled H<sub>2</sub>O, 0.1  $\mu$ l of *Taq* polymerase (Promega, buffer B), 1  $\mu$ l of primer, 1  $\mu$ l of BSA (Amresco, at 2.5 mg/ml), 5  $\mu$ l of a mastermix [500  $\mu$ l 10x reaction buffer supplied with the *Taq*, 290  $\mu$ l sterile distilled H<sub>2</sub>O, 10  $\mu$ l 1M MgCl<sub>2</sub> (Amresco), 200  $\mu$ l dNTPs (USB) at 10mM], and 1  $\mu$ l of diluted DNA. Reactions were run out on 1.5% agarose gels (Type 1, Amresco) in 0.5x TBE buffer until a bromophenol blue dye marker migrated 8 cm. Gels were stained with ethidium bromide for 20 min and destained in distilled H<sub>2</sub>O for 30 min. Gels were visualized and photographed in UV light with a Kodak EDAS 290 gel imaging system.

## Results and Discussion

A total of 40 RAPD primers (A1-20, B1-20) were surveyed for markers that distinguish *Solidago sciaphila* and *S. hispida*. RAPD markers are dominant (can not differentiate a homozygous dominant individual from a heterozygous individual) and each band is treated as a different locus, with two alleles (present or absent). Twenty-four RAPD loci are unique to either *S. sciaphila* or *S. hispida*, or at least differentiate populations (Fig. 1). It is important to score markers that identify populations because this may reveal which populations contribute alleles even if they are not in close geographic proximity. These markers will need to be evaluated further to determine their distribution in all populations sampled to date, and if they are truly unique or simply polymorphic (a population has individuals with and without the locus in question). These analyses are on-going. If hybridization is indeed occurring, we expect unique bands found in *S. sciaphila* and *S. hispida* to be found in combination in hybrid individuals. We will also investigate the utility of Inter-simple Sequence Repeat (ISSR) markers, another DNA-based genetic marker system that is able to fingerprint individuals (Wolfe et al. 1998).

## Summary

Morphologically intermediate individuals of *Solidago sciaphila* (cliff goldenrod, IL-threatened) and *S. hispida* (hairy goldenrod) have been observed in northwestern Illinois. The objective of this research was to identify genetic markers that could test the hypothesis of hybridization. RAPD markers were surveyed and 24 loci were observed to be unique to one species or the other, or at least could differentiate different populations. These markers can now be used in future work with additional populations to determine if hybridization is indeed occurring and the nature of the perceived hybridization between these two goldenrods.

Ultimately our data can be used to more effectively manage and protect the threatened cliff goldenrod.

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Table 1. Populations of *Solidago sciaphila* and *S. hispida* sampled.

Species	Abbreviation	Locality	Year of collection
<i>S. hispida</i>	TR	Galena Territories, Jo Daviess Co.	2001
mixed	CH	Council Hill, Jo Daviess Co.	2001
mixed	P1	Mississippi Palisades State Park, pop. #1, Carroll Co. North side	2001
mixed	P2	Mississippi Palisades State Park, pop. #2, Carroll Co. North side	2001
mixed	TO	Townsend Road, Jo Daviess Co.	2002
<i>S. hispida</i>	GTW	Galena Territories West, Jo Daviess Co.	2002
mixed	OPB	Oak Point Bluff, Mississippi Palisades State Park, Carroll Co. South side.	2002
<i>S. sciaphila</i>	IH	Indian Head, Mississippi Palisades State Park, Carroll Co. South side.	2002
mixed	AC	Apple Canyon State Park, Jo Daviess Co.	2002



Figure 1. Portions of RAPD gels showing candidate loci (arrows) that distinguish *Solidago sciaphila* (two different populations: S<sub>1</sub>, S<sub>2</sub>), from *S. hispida* (H). Black arrows indicate loci that differentiate the two species, while gray arrows indicate loci that may be unique to particular populations.



















