The application of genetic markers to investigate two issues concerning the conservation of *Boltonia decurrens* (Asteraceae): the genetic structure of a metapopulation and the potential for interspecific hybridization

by

Jennifer Ann DeWoody

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Program of Study Committee:
John D. Nason, Major Professor
Diane Debinski
Jonathan F. Wendel

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Graduate College
Iowa State University

This is to certify that the master's thesis of

Jennifer Ann DeWoody

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

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CHAPTER 1. INTRODUCTION

Thesis Organization

This thesis is organized into four chapters: a general introduction and literature review, two manuscripts, and a chapter describing the general conclusions of this project. Chapter 1 reviews the use of genetic marker in conservation biology and provides a literature review as a history of the federally threatened floodplain herb *Boltonia decurrens*.

Chapter 2 is a manuscript written for submittal to the journal *Conservation Biology*. This paper details the use of genetic markers to estimate demographic parameters in *B. decurrens* and to empirically test the predicted consequences of metapopulation processes in water-dispersed plants.

The third chapter is a manuscript written for submittal to the *American Journal of Botany*. This paper reports the use of genetic and morphological data to determine if and to what extent hybridization occurs between *B. decurrens* and *B. asteroides*, a widespread congener, in two sites where these species occur in sympatry.

The fourth chapter provides general conclusions for these two studies and emphasizes the implications of these results for the management and conservation of *B. decurrens*.

General Introduction

Boltonia decurrens (Torr. & Gray) Wood (Asteraceae) is an early successional, floodplain specialist endemic to a 400 km stretch of the central Illinois River floodplain and the adjoining Mississippi River. *B. decurrens* requires regular flooding in order to complete its lifecycle, (Smith & Keevin 1998; Smith et al. 1993), and habitat destruction and hydrologic changes to the Illinois River, particularly during the twentieth century, have contributed to the decline of the species (Schwegman & Nyboer 1985). In 1988 *B. decurrens* was listed as threatened under the U. S. Endangered Species Act (U.S. Fish & Wildlife Service 1988). Today, approximately 20 isolated populations remain across the species range.

Previous studies have described the life history of *B. decurrens* (Smith 1993; Smith et al. 1998). This species colonizes newly disturbed habitats, typically flooded areas devoid of standing vegetation. Populations are shade-intolerant and undergo local extinctions within

five years of establishment as later-successional species encroach upon their habitat. This frequent local colonization and extinction creates a metapopulation across the range of the species. An understanding of the affects of these metapopulation dynamics on the genetic structure and demographic processes of the species may aid in conservation efforts by providing insight to the structure of newly colonized populations, the relative amount of migration among populations, and the existence of local ecotypes across the range of the species.

In addition to the threats posed by habitat destruction and flood suppression, *B. decurrens* may be threatened by further decline in two populations where it occurs in sympatry with a widespread congener, *B. asteroides*. Hybridization may lead to a further decline of a rare species due to decreases in fertility (Levin et al. 1996), genetic swamping (Rhymer & Simberloff 1996), or increased competition or pathogen susceptibility (Soltis & Gitzendanner 1999). The ability to detect hybrids in sympatric populations and describe the structure of any resulting hybrid zones is critical to determine if management efforts are needed to maintain the genetic integrity of a rare species (Levin et al. 1996).

I used genetic techniques to investigate two issues that may be critical to the long-term persistence of *B. decurrens*: the genetic structure of the metapopulation and the potential for spontaneous hybrids to occur in natural populations. The studies were designed with three goals in mind: First, to describe the inter- and intrapopulation processes that contribute to the genetic structure observed in *B. decurrens*. Second, to determine if hybridization is a threat to the long-term persistence and genetic integrity of *B. decurrens* in the two populations where it exists in sympatry with *B. asteroides*. Third, to synthesize the conclusions of these studies into recommendations for conservation efforts.

Overview of the application of genetic markers in conservation biology

The use of genetic markers to address conservation questions has increasing in frequency over the past decades (Avise 1989; Soltis & Gitzendanner 1999). Genetic markers have been used in a variety of studies, from inferring parameters traditionally estimated using ecological or observational methods, to investigating processes inaccessible by traditional protocols. For the first category of studies, a broad range of parameters and processes can be

inferred through the use of genetic data. Several demographic processes, including migration (Slatkin 1987), mating system analyses (Clegg 1980), and relatedness (Kalisz et al. 2001) are routinely estimated from genetic data. In addition, genetic data can provide conclusive evidence of interspecific hybridization between rare and common taxa in natural systems (Rhymer & Simberloff 1996). While these processes can often be investigated using traditional demographic or field techniques, molecular data may be more appropriate for issues involving historic or evolutionary processes, or in cases where traditional techniques are not sufficient to detect the parameters of interest (e.g., low rates of migration among populations or insufficient differentiation between parental species and hybrid individuals).

In addition to complementing traditional methods, the second category of genetic applications provides opportunities to describe the structure of and processes influencing rare species that are inaccessible using ecological protocols. Phylogenetics uses molecular genetic data to infer evolutionary relationships among organisms (Avise 1994), and has provided insight into the definition and use of evolutionarily significant units (ESU) as a basis for conservation strategies (Avise 1989). Alternatively, genetic techniques can detect recent genetic bottlenecks in populations from a sample of extant individuals (Cornuet & Luikart 1996). Although demographic studies using methods such as capture-mark-recapture may detect bottlenecks in population size, the length and expense of such studies is often prohibitive, and retroactive detection is impossible.

Effects of metapopulation dynamics on population genetics

In general terms, a metapopulation consists of a population of populations (Hanski & Simberloff 1997), with each population existing in a patch of appropriate habitat surrounded by less suitable habitat. Levins' (1969) classical metapopulation model included many small, identical patches and populations in a matrix. Today, the term metapopulation can describe a variety of population structures, including a variety of patch sizes, extinction probabilities, rates of migration, and colonization mechanisms (Hanski & Simberloff 1997). Numerous theoretical studies have investigated the consequences of the frequent local extinction and colonization of patches on the demographic structure of the metapopulation (see Hanski 2001).

The theoretical consequences of frequent population turnover on the genetic structure of a metapopulation have also been investigated, (McCauley 1991; Pannell & Charlesworth 2000; Slatkin 1977; Wade & McCauley 1988; Whitlock & McCauley 1990). Models of habitat colonization can be described along a continuum between two extremes: the migrant pool model and the propagule pool model (Wade & McCauley 1988). In Slatkin's (1977) migrant pool model, colonists for a new population are drawn at random from all existing populations. This model of population colonization has been shown to decrease, maintain, or increase genetic differentiation among populations, depending on the parameters of colonization (Wade & McCauley 1988). The propagule pool model, where colonists are drawn at random from within a single population (Slatkin 1977), has been shown to increase differentiation among populations (Wade & McCauley 1988). While theoretical studies investigating the genetic consequences of frequent local colonization and extinction have focused on these two models, the modes of colonization exhibited by species in natural systems likely exist along a continuum between the two extremes (Wade & McCauley 1988).

Issues concerning metapopulations in conservation biology

Although susceptible to the same threats as species that exist in stable populations (e.g., demographic, environmental, and genetic stochasticity), rare or threatened species that persist as metapopulations present additional challenges to conservation managers (Husband & Barrett 1996). First, metapopulation theory has revealed the importance of non-occupied habitat to the persistence of a species, where previous management efforts may have focused entirely on occupied patches (Hanski 1998; Hanski & Simberloff 1997). Second, incorporating metapopulation theory into conservation strategies requires the maintenance of migration among habitat patches (Schemske et al. 1994), which may vary with patch density or require habitat corridors (Hanski et al. 1996). Third, although the genetic consequences of frequent local extinction and colonization have been investigated theoretically, two issues of great potential to conservation strategies, the effective size of a metapopulation and the minimum viable metapopulation size, have received less attention (but see (Hanski et al. 1996; Hedrick & Gilpin 1997).

Genetic techniques provide an opportunity to investigate the importance of specific processes to the persistence of a metapopulation. Estimates of genetic variation within and among populations can be used to infer historic migration rates among populations (Slatkin 1987), detect recent bottlenecks in populations (Cornuet & Luikart 1996), and describe the effects of frequent colonization (McCauley et al. 1995; Ims & Yoccoz 1997). Specifically, genetic markers can be used to describe the process of population colonization in terms of the propagule pool and migrant pool models. Given the absence of inbreeding, high levels of genetic differentiation among populations within a metapopulation are expected to result from colonization processes similar to the propagule pool model, while low levels of differentiation among populations indicate that new populations are formed by processes similar to the migrant pool model (see Wade & McCauley 1988). Empirical analyses testing these predicted consequences of metapopulation processes are lacking, especially in plant species (Husband & Barrett 1996; Giles & Goudet 1997).

Implications of hybridization to conservation biology

Although interspecific hybridization has been shown to be widespread among angiosperms (Rhymer & Simberloff 1996) and may serve as a mechanism of speciation in natural populations (Grant 1981), hybridization between rare and common taxa may have deleterious consequences for the threatened species (Whitham & Maschinski 1996). Hybridization may lead to the further decline of a threatened species through decreased fertility (Levin et al. 1996), genetic swamping (Rhymer & Simberloff 1996; Whitham & Maschinski 1996), or increased competition with hybrid individuals (Soltis & Gitzendanner 1999). These threats are particularly important if the threatened species is severely reduced in numbers (e.g., Catalina mahogany, Rieseberg 1991) or if introgression occurs asymmetrically between species, (e.g., Louisiana irises, Arnold et al. 1991), potentially threatening the genetic integrity of the rare taxon. In order to develop sufficient management protocols to counter the effects of interspecific hybridization, it is necessary to detect any hybrids in natural populations, and describe the structure of any hybrid zone that may be formed.

Genetic markers can provide conclusive evidence of interspecific hybridization in natural populations (Rhymer & Simberloff 1996; Rieseberg et al. 1998). Previous studies have demonstrated that hybrid individuals can be identified using genetic markers given a number of unique, or diagnostic alleles in the parental species (Nason et al. 1992). Recently, Nason et. al (in press) described techniques that do not require unique alleles to detect first- and second-generation hybrid progeny at sympatric sites. These techniques can aid in management efforts by providing empirical evidence of the potential for and extent of hybridization in natural populations.

Boltonia decurrens, a federally threatened floodplain herb

Boltonia decurrens (T. & G.) Wood (Asteraceae) is an early-successional floodplain specialist, restricted to the Illinois River and the adjoining Mississippi River. *B. decurrens* relies on regular flooding events to complete its lifecycle. Flowers are produced in the late summer and early fall (Stoecker et al. 1995). Both ray and disc flowers are fertile, and produce dimorphic achenes. Ray achenes are small (1.3 mm x 0.9mm), three-sided, and lack substantial awns (Smith & Keevin 1998). Disc achenes are also small (1.8 mm x 1.3 mm), flat, and posses two long awns shown to aid in flotation (Smith & Keevin 1998). As an inflorescence may produce six times as many disc flowers as ray flowers (Morgan 1966), water dispersal (hydrochory) of the disc achenes is believed to be the major mechanism of dispersal by the species (Smith & Keevin 1998).

Achenes require high light and moisture conditions to germinate (Smith & Keevin 1998), and germination may take place in the fall or the following spring. Seedlings developing in the fall overwinter as rosettes and bolt and flower the following spring. Seedlings that become established in the spring can bolt and flower the same year. Flowering stalks become 1.5-2 m in height and produce a branching inflorescense (Stoecker et al. 1995). Plants are prolific seed producers, estimated to procude ca. 50,000 achenes per year (Smith & Keevin 1998).

In addition to sexual reproduction, *B. decurrens* is capable of vegetative reproduction via basal rosettes (U. S. Fish & Wildlife Service 1990). In the fall each flowering stem will produce between five and seven, but up to eleven, basal rosettes, creating a clumped

distribution of ramets in established populations (U. S. Fish & Wildlife Service 1990). Successive establishment of basal rosettes around a mother stem can lead to the death of a genet due to decreased light and nutrient availability (Smith, pers. comm.).

Boltonia decurrens is an early successional species, establishing new populations in areas recently cleared of vegetation by flooding disturbances (Smith & Keevin 1998). Plants are poor competitors due to the species' high light requirements (Smith et al. 1993), and populations decline within three to five years of establishment due to encroachment by later-successional competitors (Smith & Keevin 1998). Frequent local extinction and colonization events create a metapopulation of isolated populations across the range of the species.

Once thought to constitute a contiguous population along a 400 km stretch of the Illinois and adjoining Mississippi Rivers, habitat destruction contributed to the decline of *B. decurrens* over the twentieth century (Schwegman & Nyboer 1985). Historically, seasonal, moderate floods were common on the Illinois River (Bellrose et al. 1983). Levee systems and other flood control measures have suppressed the flooding required by the species for dispersal and germination (Smith et al. 1998), and the conversion of floodplain habitat to agricultural or urban areas, have reduced the amount of habitat available to new populations of *B. decurrens* (Schwegman & Nyboer 1985). This decline of the species led to the first recommendation for its protection under the U.S. Endangered Species Act (U.S. ESA) in 1974 (U. S. Fish & Wildlife Service 1988). However, various political and taxonomic events, including the elevating of the taxon from a variety to species status by Schwegman & Nyboer (1985, see *Taxonomic history*, below), prevented the listing of the species until 1988 (U. S. Fish and Wildlife Service 1988). Extensive surveys to locate extant populations of *B. decurrens* were completed by Schwegman & Nyboer (1985) prior to the species' listing under the U.S. ESA identified eight isolated populations, all in areas regularly disturbed by human activities. Several populations were established or regenerated following the severe floods of 1993 (on the Mississippi River) and 1995 (on the Illinois River), but successive years of average or below-average flood levels have resulted in a net decline of populations (pers. obs.).

The recovery plan for *B. decurrens* developed by the U. S. Fish & Wildlife Service (1990) identified a variety of objectives for the protection of the species, including mandates

for further surveys, research into population requirements and attempts to establish new populations in suitable habitat. Previous studies have investigated germination requirements and dispersal potential of achenes (Smith et al. 1995; Smith & Keevin 1998), light and soil requirement of plants (Smith & Moss 1998), and the effects of long term flooding on the *B. decurrens* and its competitors (Smith et al. 1998; Smith & Moss 1998). Current management efforts use discing, fire, and other mechanical techniques to simulate flooding disturbance, with little success. No attempts have yet been made to establish novel populations of *B. decurrens*, however, and little is known of the structure of newly colonized populations under natural conditions.

A history of *B. decurrens* and *B. asteroides*, a widespread congener

The genus *Boltonia* is native to North America and contains five species (Anderson 1987). A review of the taxonomic history of the genus is provided in Morgan (1966), Anderson (1987), and by the U. S. Fish & Wildlife Service (1988). This study is concerned with the potential for hybridization between *B. decurrens*, a species restricted to Illinois and Missouri river bottomlands, and *B. asteroides,* a congener known to occur across the eastern United States (see Figure 1), and will restrict the review of this genus to these two species as a result.

Boltonia asteroides (L.) L'Her. was first described in 1767 under the genus *Matricaria.* After being transferred to the genus *Boltonia* by L'Heritier de Brutelle in 1788, a number of additions were made to the genus, including that of a variety of *B. asteroides* described as *decurrens* by Torrey and Gray (Morgan 1966). Wood later raised this variety to specific rank (Morgan 1966). The first comprehensive treatment of the genus was not completed until Fernald (1940, in Morgan 1966). Several treatments were proposed over the next forty years (see Anderson 1987), all of which considered *B. decurrens* as a variety of *B. asteroides*. Schwegman & Nyboer (1985) elevated *B. decurrens* to specific status once again based on leaf morphology (copious decurrence is present in *B. decurrens* but absent in *B. asteroides*) and root morphology (lack of rhizomes in *B. decurrens*).

Morgan (1966) completed a thorough morphological, anatomical and cytological investigation of the genus *Boltonia*, treating *B. decurrens* as a variety of *B. asteroides*.

Although her work is unpublished, it identified a morphological difference between the two taxa which was not investigated by Schwegman & Nyboer (1985): significant variation in flower number per inflorescence. In addition, no study has used molecular genetic techniques to investigate the relationship between these species. Such information as genetic differentiation and genetic distance may provide insight into the long debated taxonomic relationship of these species.

Currently, only two sympatric populations of *B. decurrens* and *B. asteroides* are known to exist. Although previous studies at sympatric populations determined that hybridization between the two species was unlikely (Schwegman & Nyboer 1985), recent field observations have identified intermediate morphologies indicative of spontaneous hybrids (pers. obs., P. Mettler, pers. comm.). The possibility of spontaneous hybridization between *B. decurrens* and *B. asteroides* may have implications not only for the taxonomic status, but also the protected status of *B. decurrens*. Historically, the U. S. ESA prohibited the protection of species known to hybridize with common taxa, but current interpretations of the policy allows the listing of threatened taxa if hybrid progeny more closely resemble the listed parent than the intermediate of the two species (Rhymer & Simberloff 1996; Soltis & Gitzendanner 1999; Whitham & Maschinski 1996). Fully understanding the potential for and extent of hybridization between these species under natural conditions is critical to the protection of *B. decurrens*.

Objectives of study and implications for conservation

I used neutral genetic markers (allozymes) to investigate two processes critical to the long-term survival of *B. decurrens*: the role of metapopulation dynamics in determining the genetic structure of the species, and the potential for recurrent hybridization between *B. decurrens* and a widespread congener, *B. asteroides*. Understanding the metapopulation dynamics and demographic processes influencing the genetic structure of this species can provide critical information to guide future management decisions. I determine if two processes critical to the life history of the species, the potential for hydrochory (seed dispersal via water) to result in unidirectional gene flow and the predicted consequences of frequent colonization and extinctions, significantly influence the genetic structure of the

metapopulation. I investigate whether hydrochory (seed dispersal by water) has led to unidirectional gene flow among populations by asking three questions. First, does gene flow among populations decrease as a function of distance? Second, is the genetic variation non-randomly distributed among populations? Specifically, do downstream populations contain higher levels of genetic diversity than upstream populations? Third, are upstream populations ancestral to downstream populations?

I test for evidence of frequent local population extinction and recolonization in *B. decurrens* by asking three additional questions. First, is the genetic differentiation among populations indicative of the migrant pool model of colonization? I predict that mixing of seed along the river should be panmictic, resulting in low or zero levels of genetic differentiation among populations. Second, have populations of *B. decurrens* recently undergone genetic bottlenecks? Third, is there evidence of annual differences in allele frequencies within single populations?

The ability of *B. decurrens* to hybridize with *B. asteroides* may influence the viability of *B. decurrens* where the two species occur in sympatry. I conducted morphological and genetic analyses in order to determine whether hybridization occurs under natural conditions. First, I ask whether morphological differences between putatively pure parental species populations permit the identification of hybrid individuals. Second, I characterize allozyme allele frequencies for each species and use likelihood methods to determine the power of these markers to distinguish parental species, as well as any first- and second-generation hybrid progeny. Third, I use the most diagnostic morphological characteristics and the allozyme markers, both separately and jointly, to estimate the frequency of hybridization and to identify hybrid individuals in two sympatric populations. Finally, I discuss the occurrence, extent, and asymmetry of hybridization between these species, and its implications for the management and preservation of *B. decurrens* populations as well as its classification under the U.S. Endangered Species Act.

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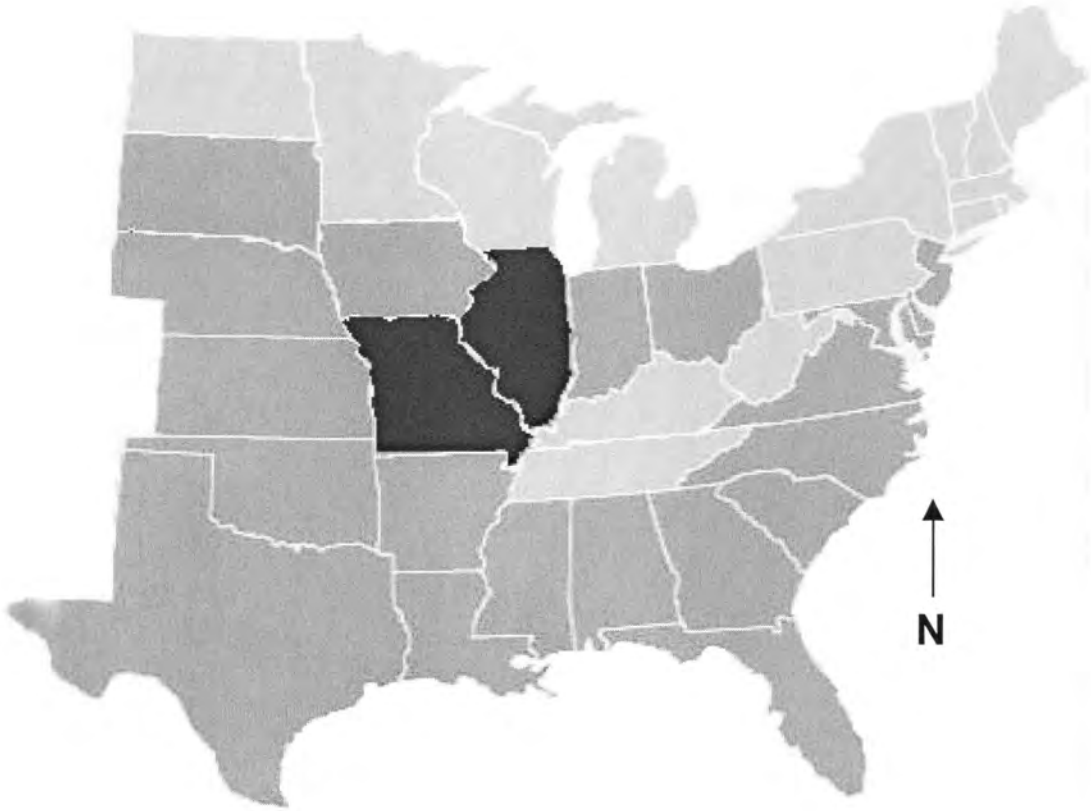

Figure 1. Distribution of *Boltonia decurrens* and *B. asteroides*. Black denotes the presence of both *B. decurrens* and *B. asteroides* (IL and MO). Dark gray indicates the presence of *B. asteroides*. Light gray denotes states lacking either species.

CHAPTER 2. MULTIPLE DEMOGRAPHIC PROCESSES INFLUENCE THE GENETIC STRUCTURE OF *Boltonia decurrens* (ASTERACEAE), A THREATENED FLOODPLAIN SPECIES

A paper prepared for submission to *Conservation Biology*

J. A. DeWoody, J. D. Nason and M. Smith

Abstract

As a floodplain specialist, *Boltonia decurrens* (Asteraceae), a federally threatened herb endemic to the Illinois River floodplain, requires regular flooding events for generation of suitable habitat and for seed dispersal. Flood suppression and habitat destruction during the twentieth century have contributed to the species' decline and the fragmentation of remaining habitat into isolated patches. *B. decurrens* is currently restricted to fewer than 25 populations along a 400 km stretch of the Illinois River and individual populations frequently undergo local extinctions and recolonizations. The isolation of populations by tens of km likely results in gene flow among populations being limited to seed dispersal during flooding events. We used neutral genetic markers to determine how inter- and intra-population processes contribute to the genetic structure of *B. decurrens*. Samples were collected from fourteen populations of *B. decurrens* across the range of the species and assayed for allozyme variation. Significant genetic differentiation was detected among populations (F_{ST} = 0.098, 95% CI: 0.080, 0.114), indicating that colonization events involve propagules from a small number of extant populations. We tested the hypothesis that hydrochory results in unidirectional gene flow among populations, but found no evidence that genetic variation is greater in downriver populations. Tests of isolation by distance found no evidence that gene flow decreases as a function of distance revealed that hydrochory (seed dispersal by water) contributes to long-distance migration and/or population founding events. A variety of intrapopulation processes were found to contribute to the average fixation index within populations (F_{IS} = 0.192, $p < 0.05$). A mating system analysis indicates that the mean outcrossing rate ranged from 0.6 to 1.0 within populations. Spatial autocorrelation analyses

detected no fine-scale population structure in two populations, suggesting the significant F_{IS} values may be the result of selfing but not biparental inbreeding. Recent genetic bottlenecks, detected for 12 of 14 populations surveyed, may also contribute to the fixation index. Together, the levels of genetic differentiation observed within and among populations of *B. decurrens* are consistent with the patterns expected as a result of frequent population extinctions and colonization events. Regular, severe flooding events are necessary to maintain these processes and insure the persistence of the metapopulation.

Introduction

Although susceptible to the same threats as species that exist in stable populations (e.g., demographic, environmental, and genetic stochasticity), rare or threatened species that persist as metapopulations present additional issues to be addressed by conservation managers (Husband & Barrett 1996). The ephemeral nature of subpopulations within a metapopulation emphasizes the importance of unoccupied habitat to the survival of the species (Hanski & Simberloff 1997; Hanski 1998). Additionally, successful management strategies for a metapopulation require a better understanding of landscape-level processes (e.g., dispersal and migration) than do those for single-population efforts. Ultimately, an understanding of the factors influencing the colonization and extinction of subpopulations is required for successful conservation of a metapopulation (Schemske et al. 1994). Understanding the genetic dynamics of the metapopulation structure (e.g., relatedness of founders of new populations or the historic migration between populations) may also aid in conservation efforts by providing insight into parameters required for the survival of the metapopulation, such as the minimum viable metapopuations size or the minimum density of sustainable habitat patches (Hanski et al. 1996). Few studies, however, have empirically tested the predicted genetic consequences of the frequent local colonization and extinction associated with metapopulation dynamics (McCauley et al. 1995; Husband & Barrett 1996; Giles & Goudet 1997; Thrall et al. 1997).

The theoretical consequences of recurrent local colonization and extinction events on the genetic structure of a metapopulation have been well established (Pannell & Charlesworth 2000). (Slatkin 1977) depicted two extreme models describing the genetic

effects of extinction and colonization within a metapopulation: the migrant pool and propagule pool models. In the migrant pool model, founders of a new population have an equal probability of being drawn from all populations within the metapopulation. Wade & McCauley (1988) showed that this mode of colonization could either increase or decrease population differentiation relative to initial conditions depending on the parameters governing colonization. In the propagule pool model, the founders of a new population are all chosen at random from a single source population (Slatkin 1977). Wade & McCauley (1988) showed that this mode of colonization increases population differentiation among the metapopulation. Whitlock & McCauley (1990) generalized the propagule pool model to permit the founders of a new populations to be related (e.g., seeds within a single fruit). This kin-biased colonization was shown to significantly increase allelic variance among populations (Whitlock & McCauley 1990). Although the actual process of extinction and colonization in a natural metapopulation is expected to fall along a continuum between migrant and propagule pool models (Wade & McCauley 1988), placing the colonization process in a threatened metapopulation in the context of these models may provide insight into critical demographic processes which could be applied to future conservation or restoration efforts. Moreover, the ability to generalize these processes across species displaying similar life histories may aid in the restoration of floodplain plant communities as a whole.

Boltonia decurrens (Torr. & Gray) Wood (Asteraceae), a federally threatened herb, is native to the Illinois River floodplain and a short section of the Mississippi River at the confluence of the two waterways. *B. decurrens* relies on regular flooding not only for seed dispersal (Smith & Keevin 1998), but also for the generation of acceptable habitat for new populations (Schwegman and Nyboer 1985; U. S. Fish & Wildlife Service 1990; Smith et al. 1998). *B. decurrens* is an early-successional species, and established populations are susceptible to extinction within 3-5 years of establishment as later-successional species encroach upon the population (Smith et al. 1993). This frequent local extinction and colonization of populations creates a metapopulation (as defined by (Hanski & Gilpin 1991), but see (Hanski & Simberloff 1997) along the Illinois River. Once thought to constitute a contiguous population along a 400 km stretch of the Illinois River, *B. decurrens* has declined

during the past century due to the channelization of the river (Bellrose et al. 1983), as well as urban and agricultural development across the river floodplain (Schwegman & Nyboer 1985; Service 1990). Currently 23 populations have been documented and are monitored. Previous studies have focused on the demography, physiology and life history of the species (e.g., Smith & Keevin 1998; Smith & Moss 1998), as well as those factors contributing to the local extinction of populations (U. S. Fish & Wildlife Service 1990; Smith et al. 1993), but a greater understanding of the inter-population dynamics of this species, such as processes governing the establishment of new populations, would aid conservation and restoration efforts currently underway (Smith 1994).

In addition to metapopulation dynamics, unidirectional gene flow downstream due to hydrochory (seed dispersal by water) may have a significant impact on the genetic structure of *B. decurrens* populations. Previous studies have shown that hydrochory can result in long distance seed dispersal events among populations (Kudoh & Whigham 1997; Akimoto et al. 1998). In addition, the tendency for gene flow to occur in a downstream direction can also result in the non-random distribution of genetic diversity among populations due to the greater potential for novel alleles to migrate into downstream populations (Akimoto et al. 1998; Gornall et al. 1998). The history of recurring local extinction and colonization of populations of *B. decurrens* coupled with the potential for unidirectional gene flow due to hydrochory indicates that a variety of factors may be influencing the genetic structure of the metapopulation.

Given the life history of *B. decurrens*, we predict that both metapopulation dynamics and unidirectional gene flow will significantly influence geographic patterns of genetic variation in the species. We evaluated these hypotheses using a battery of putative neutral genetic markers (allozymes). We tested for the genetic consequences of unidirectional gene flow by addressing two questions: Do downstream populations contain higher levels of genetic diversity than upstream populations, and, are upstream populations ancestral to downstream populations? We tested for evidence of local extinctions and colonizations by asking two additional questions: First, is genetic differentiation among populations indicative of the migrant pool or propagule pool models of colonization? Given the history of moderate to severe flooding along the Illinois River and the potential for backwaters up to

160 km (100 miles) or more to develop from the mouth of the Mississippi (Kofoid 1903), it is conceivable that flooding events result in large dispersal events, with seeds from many populations mixing on the river current. From this model of seed distribution, we predict that colonization events will be more consistent with the migrant pool model than the propagule pool model. As a result, levels of genetic differentiation among populations should be low and exhibit little evidence of declining as a function of distance between populations. Second, are patterns of allelic variation in contemporary *B. decurrens* populations indicative of recent genetic bottlenecks? Such bottlenecks are expected to indicate small effective population sizes resulting from founding events or population senescence.

Materials and Methods

Study species

B. decurrens is an early-successional species that colonizes newly cleared habitat following flooding events. Seeds require light and saturated, sandy soil to germinate, and recruitment of seedlings is low within a population (Smith & Keevin 1998). Individuals are shade-intolerant, resulting in the local extinction of populations within five years in the absence of further disturbance events. This recurrent colonization and extinction results in a population structure best described as a metapopulation. Seedling rosettes bolt in the spring either the same year as germination or the following season. Basal rosettes, produced at the base of each bolting stem each fall, remain green over winter and bolt the following spring. Bolting stems are prolific seed producers, averaging ca. 50,000 achenes per plant (Smith & Moss 1998). Successive establishment of basal rosettes around a mother stem will eventually lead to the death of the genet due to competition for resources (Smith, pers. observation), resulting in an average lifespan of 5-7 years per genet.

Approximately 20 populations of *B. decurrens* are known to occur along a 400 km stretch of the Illinois River and adjoining Mississippi River. Due to the ephemeral nature of *B. decurrens* populations, the precise location and size of each population varies from year to year. Fourteen populations were sampled for this study (see Table 1). Samples were also collected from one population of *Boltonia asteroides*, a widely distributed congener of *B. decurrens*, as an outgroup for phylogenetic analysis (see Table 1).

Allozyme analysis

Samples for allozyme analysis were collected in the field in May 2000, May 2001, or September, 2001. Approximately 0.06 g of leaf tissue was collected from each individual and frozen in liquid nitrogen. All samples were stored at -80°C until processed. Total protein extraction took place by macerating samples in 0.5 mL of crushing buffer (Phosphate Buffer of Soltis et al. (1983) modified to contain 6% w/v PVP), then absorbing the solution onto 3 mm X 8 mm Whatman® paper wicks. Wicks were stored at –80° C until electrophoresis.

All electrophoresis took place in 10% w/v horizontal starch gels in one of three buffers: morpholine citrate at pH 6.1 (MC6.1) and pH 7.2 (MC7.2) (from Murphy et al. 1996), or lithium hydroxide-borate "System 8" (S8, modified from Soltis et al. 1983). Samples were assayed for 11 protein stains, resulting in 13 putative loci. Seven loci were resolved in MC6.1: malic enzyme (ME1, EC 1.1.1.40), 6-phosphogluconate dehydrogenase (PGD2 and PGD3, EC 1.1.1.44), phosphoglucoisomerase (PGI1, EC 5.3.1.9), shikimic dehydrogenase (SKDH1, EC 1.1.1.25), and triose-phosphate isomerase (TPI1 and TPI2, EC 5.3.1.1). Three loci were resolved in MC7.2: isocitrate dehydrogenase (IDH1, EC 1.1.1.42), phosphoglucomutase (PGM1, EC 5.4.2.2), UTP-glucose-1-phosphate uridylyltransferase (UGPP1, EC 2.7.7.9). Three loci were resolved in S8: diaphorase (DIA1, EC 1.8.1.4), menadione reductase (MNR1, EC 1.6.99.2), and fluorescent esterase (FE1, EC 3.1.1.-). All stain recipes were adapted from Wendel & Weeden (1989) except UGPP (Manchenko 1994). All loci produced banding patterns consistent with published protein structures and diploid, Mendelian inheritance.

Analysis of population genetic structure

Quantification of within population genetic diversity

In order to quantify the genetic structure of *B. decurrens* across the range of the species, samples were collected from fourteen populations (see Table 1 and Figure 1). Genetic variation was measured in terms of the percent polymorphic loci (P), average number of alleles per locus (A), number of alleles per polymorphic locus (Ap), expected

equilibrium heterozygosity (H_e). We also calculated the fixation index (F) for each population (a measure of excess homozygosity relative to Hardy-Weinberg proportions).

Tests of hierarchical genetic structure among populations

We used Wright's F-statistics to test two *a priori* predictions of hierarchical population structure in *B. decurrens* based on the distribution of populations along the Illinois and Mississippi Rivers. The first hierarchical assumes no regional structure among populations, resulting in a 2-level hierarchy that estimates the fixation index within populations (F_{IS}) and allelic variation among populations relative to the total metapopulation (F_{ST}). The second model assumes a three-level hierarchy, with populations clustered into two regions: a northern region located on the upper Illinois River (populations 1 thru 9), and a southern region located on the lower Illinois and adjoining Mississippi Rivers (populations 10 thru 14). For this three-level model we estimated the fixation index within populations (F_{IS}), allele frequency variation among subpopulations within regions (F_{SR}) and among regions relative to the total metapopulation (F_{RT}). Only if F_{RT} differed significantly from zero did we reject the 2-level hierarchical model in favor of the 3-level model. F-statistics were estimated using the methods of Weir & Cockerham (1984) as implemented by the program GDA (Lewis & Zaykin 2001). The significance of these estimates was determined by bootstrapping over loci (1000 replicates).

Tests for isolation by distance

Previous studies have demonstrated that hydrochory can result in long-distance gene flow events (Kudoh & Whigham 1997). We tested for isolation by distance using the methods of Slatkin (1993) and Rousset (1997). As the results of the two tests do not differ, we describe and present only the Slatkin (1993) analysis. Slatkin (1993) showed that when gene flow decreases as a function of distance, the effective number of migrants between populations ($M\hat{}$) is inversely proportional to the geographic distance between populations on a logarithmic scale. In addition, Slatkin (1993) demonstrated that the expected relationship between $M\hat{}$ and distance differs between two models of gene flow. For the case of gene flow occurring under isolation by distance in a one-dimensional stepping stone manner, the

regression of distance on M^ on a log scale will produce a slope of –1. For the two-dimensional stepping stone model under isolation by distance, a slope of –1/2 is produced. In this manner we can test which model of gene flow best describes seed dispersal in *B. decurrens* by comparing the slope of any significant regression to those expected values. Based on the linear arrangement of populations of *B. decurrens* along the Illinois River, we expect this association to follow the one-dimensional stepping stone model if gene flow decreases as a function of distance. M^ (an estimate of *Nm*) was calculated from pairwise F_{ST} values estimated using the method of Nei (1972) for all possible pairs of populations (Slatkin 1993). We tested for isolation by distance under two distance models: direct distance (km) between populations (as calculated using a Garmin® handheld GPS unit) and river distance (km) between populations (as estimated using 1:150,000 scale maps in the Illinois Atlas and Gazeteer, Delorme, 2000). Significance of each correlation was tested with randomization tests implemented using the program Permute! (http://www.fas.umontreal.ca/BIOL/Casgrain/en/labo/permute/index.html).

Tests of unidirectional gene flow due to hydrochory

Tests for non-random distribution of genetic variation

Unidirectional gene flow due to hydrochory has been shown to result in non-random distribution of allelic variation among populations (Gornall et al. 1998). We expect downstream populations to contain greater amounts of genetic variation due to the greater potential for novel alleles to migrate along river currents. We test the hypothesis that upstream populations contain lower amounts of genetic variation than downstream populations by regressing the distance (km) of each population from the southernmost population (#14) against four measures of genetic diversity: average number of alleles per polymorphic locus (AP), percent polymorphic loci (P), and expected (H_e) and observed (H_o) heterozygosity for each population. Both direct distance and river distance from population 14 (estimated as described above) were tested in the regression models. The significance of each regression model was tested using JMP (version 4.0.4, SAS Institute, 2000).

Evolutionary relationships among populations

We also tested for asymmetrical hydrochory by estimating evolutionary relationships among populations. Unidirectional seed dispersal may result in upstream populations contributing significantly more seed, than populations located downstream, to the establishment of new populations. This asymmetry is expected to result in a hierarchically nested population phenogram, in which upstream populations are ancestral to downstream populations (see Figure 2A). We constructed a population phenogram for the 14 study populations using Nei's (1972) genetic distance and neighbor-joining methods. Support for the observed tree was determined via bootstrap analyses using 1000 replications. All procedures for genetic distance estimation, neighbor-joining, and bootstrapping were completed using the program PHYLIP (Felsenstein 1993).

The topology of the observed phenogram was tested for concordance with the hypothetical phenogram using the program GeneTree (Page 1998). This program estimates the number of duplications or losses, or for this scenario the number of extinctions or unsampled populations, necessary to reconcile the hypothetical cladogram with the observed phenogram. To determine the distribution of the test statistic for our data set, we generated 1000 random cladograms simulated using the Yule (Markovian) model. The test statistic associated with the observed data (phenogram) was considered significantly different from random, and not significantly different from the hypothesized phenogram, if less than the 50^{th} lowest ranked value of the null distribution ($\alpha = 0.05$).

Examining the consequences of frequent local extinctions

Tests for recent population bottlenecks

As new populations are founded or older populations tend toward local extinction, the small effective size of the population may result in a genetic bottleneck. We tested for recent genetic bottlenecks in all populations using the method described in Cornuet & Luikart (1996). This procedure tests for significant difference between the Hardy-Weinberg heterozygosity (H_e) and the level of heterozygosity expected in a population at mutation-drift equilibrium (H_{eq}). H_e is calculated from sample allele frequencies and is relatively insensitive to low frequency alleles. H_{eq}, in contrast, is calculated from the total observed

number of alleles, giving equal weight to all alleles, regardless of differences in frequency. As a result, bottlenecks are expected to result in a transient disequilibrium between H_e and H_{eq} (see Cornuet & Luikart 1996; Luikart & Cornuet 1998; Piry et al. 1999). We tested for evidence of recent bottlenecks in each population using the one-tailed Wilcoxon's test for excess heterozygosity under the infinite alleles model as implemented in the program Bottleneck (Cornuet & Luikart 1996).

Mating system analysis

Significant deficits of heterozygosity within a population may be produced by significant self-fertilization or by other population-level processes, including correlated colonization similar to the propagule pool model of population establishment. In order to determine if the mating system of natural populations of *B. decurrens* contributes to any excess of homozygosity observed within populations, progeny arrays were grown in the Iowa State University Bessey Greenhouse during the fall of 2001. Seed for these progeny arrays was collected from three populations (nos. 1, 9, and 10) in 1995. Progeny arrays consisted of ten progeny (seedlings) from each of ten mothers (one capitula per mother) for each population. Fresh tissue was assayed for allozyme variation as described above for a subset of loci: PGI1, SKDH1, PGD3, ME1, TPI1, FE1, DIA1, and MNR1. (Ritland 2002) multilocus approach was used to estimate the average outcrossing rate per population (t_m) and the correlation of paternity among outcrossed sibs (r_p). Based on previous hand pollination experiments showing that *B. decurrens* is capable of low amounts of self-fertilization (5% viable seed), we expected t_m to be no different from 1.0. The correlation of paternity (r_p) estimates the proportion of full sibs relative to the total outcrossed progeny in a population (Brown 1989).

Tests for fine-scale population structure

Fine-scale population substructure, where neighbors are more closely related than expected at random, results from localized seed dispersal, which, coupled with localized pollen dispersal, may lead to biparental inbreeding and excess homozygosity within a population. Coupling the spatial autocorrelation analyses with the mating system analysis

permits us to resolve the relative roles of self-fertilization or near-neighbor inbreeding within populations of *B. decurrens*. A significant excess of homozygosity, estimated by F_{IS}, with no evidence of selfing or biparental inbreeding, or spatially structured populations, implicates other population-level processes, such as a Wahlund effect at population founding.

We tested for fine-scale population substructure in two populations (nos. 5 and 13) of *B. decurrens* by collecting 60 samples at 1-m intervals along a transect. Two sets of samples were collected at population 5, one in May 2000 and one in May 2001. One set was collected from population 13 in May 2001. The multilocus genotype for each individual was determined using the allozyme assays described above. Spatial autocorrelation analyses using Nason's multilocus estimator of kinship (Kalisz et al. 2001) were used to test for fine-scale population structure in each sample set. This method uses bootstrap analyses to estimate the 95% confidence interval around the null hypothesis of no fine-scale population structure. Kinship coefficients exceeding this confidence interval at near-neighbor distances indicate the significant fine-scale population structure required for biparental inbreeding to occur.

Examining the genetic consequences of frequent colonizations

Due to the difficulty of tracking seed from individual populations during dispersal events and the absence of newly established populations during the study period, we indirectly describe the process of population colonization in *B. decurrens* by addressing two separate questions. First, as an initial test of whether population establishment is better described by the propagule pool model or the migrant pool model of colonization, we infer the number of populations that contribute to colonization events (see *Intrapopulation cluster analysis* below). Second, in order to determine the potential for seed to migrate along the river, we infer the origin of founders by completing an admixture analysis for each cluster of individuals identified in the cluster analysis. From these results we can infer the contribution of individual populations, and thus the distance traveled by seed, during colonization or migration events (see *Admixture analysis* below). We completed these tests for three populations first reported after the large-scale census of *B. decurrens* in 1984: nos. 4 (first reported in 1989), 12 (reported in 1994), and 13 (reported in 1994). Ninety-six samples were

randomly collected from each population in May 2001. The multilocus genotype of each sample was determined using the methods described above.

Intrapopulation cluster analysis

If more than one population contributes founders to colonization events, and sufficient differentiation exists between founder populations, the resulting population will display substructure characteristic of the different sources of propagules. That is, the new admixed population will be composed of subpopulations from each source population, in proportion to the amount of propagules contributed by each. In the case of *B. decurrens*, the possibility of random mating subsequent to population establishment means that admixed individuals, potentially containing genes from multiple source populations, may be present in the admixed population. (Pritchard et al. 2000) developed a clustering algorithm that uses Bayesian methods to estimate the likelihood that a defined number of subpopulations exist in a population sample, and to assign individuals to each cluster based on their multilocus genotype. We inferred the most likely number of subpopulations present in each population using the procedure described by (Pritchard et al. 2000). We tested the likelihood that k subpopulations exist in each population for all $k = \{1, \ldots, N\}$, where N is the number of clusters such that $k > N$ results in consistently decreasing likelihoods. N was determined by initial, unreported tests. We also used the procedure to assign individuals from each test population to the k subpopulations identified as most likely by the cluster analysis.

Admixture analysis

In order to determine the distance traveled by seed during colonization events, we used an admixture analysis to assign individuals from each of the subpopulations defined in the cluster analysis (above) to their population of origin. The set of putative source populations (S) was defined as those populations from the sample set known to be extant at the time each test population was first reported (see Table 1): for population 4, $S = \{1 - 3, 5 - 11, 14\}$; for populations 12 and 13, $S = \{1 - 11, 14\}$. The admixture software GeneClass (Cornuet et al. 1999) assigns individuals to putative source (or reference) populations based on their multilocus genotypes using a variety of distance or likelihood methods. Since it is

not known which model most accurately describes genetic differentiation in *B. decurrens*, we tested the methods of population assignment based on Bayesian likelihood, Nei's standard genetic distance, and Cavalli-Sforza distance using simulated populations of 10,000 individuals created based on observed allele frequencies at putative source populations. The most appropriate model, defined as the model with the highest percent of individuals correctly assigned to their source population averaged over simulated populations, was used in the data analysis.

Population assignment tests for each of the three test populations were completed using the most appropriate model based on the simulation tests. We determined the null distribution of the test statistic for each of the test populations by using the simulation procedure in GeneClass (Cornuet et al. 1999). We simulated 10,000 individual test statistics for each of the putative source populations, and defined the critical value for assignment for that population to be the (α*10,000)th test statistic in that distribution. The critical value for each individual test was determined by adjusting an experiment-wise error rate of α=0.05 using the Dunn-Sidak method (Sokal & Rohlf 1995). Any individual assigned to a source population with a probability less than the adjusted alpha was removed from the analysis.

Tests for annual differences in population structure

The ephemeral nature of *B. decurrens* populations may result in annual variation in allele frequencies within populations due to local population bottlenecks or recent gene flow events. To test for annual differences in allele frequencies we collected samples from four populations (nos. 5, 7, 9, and 12) in both May 2000 and May 2001. The allele frequencies estimated for the two collections were used to calculate F_{ST}, the allele frequency variation between years for each site. Significant F_{ST} values across years would be consistent with the genetic structure expected for highly ephemeral populations. F_{ST} values were estimated using the methods of Weir & Cockerham (1984) as implemented by the program GDA (Lewis & Zaykin 2001). The significance of these estimates was determined by bootstrapping over loci (1000 replicates).

Results

Analysis of population genetic structure

Quantification of within population genetic diversity

Eleven of thirteen allozyme loci (84.6%) surveyed were variable with high levels of genetic diversity maintained within individual populations of *B. decurrens* (Table 2). No population contained unique alleles, although several alleles were present in a limited number of populations, including FE1-8 and PGM1-2, each observed in only three populations (see Appendix). In addition, seven populations (nos. 1, 4, 5, 8, 10, 12, and 13) exhibited an excess of homozygotes, as indicated by significant fixation indices (F_{IS}; mean 0.192, Table 2).

Tests of hierarchical genetic structure among populations

Significant differentiation among populations of *B. decurrens* was observed in both the 2- and 3-level hierarchical models (Table 3). Differentiation among regions was not significant for the two-region model (F_{RT} = 0.013; 95% CI: -0.004, 0.034), however, and variation among populations within regions (F_{SR} = 0.104; 95% CI: 0.081, 0.125) was consistent with the variation observed among all populations in the two-level model (F_{ST} = 0.098; 95% CI: 0.080, 0.114).

Tests for isolation by distance

No evidence for isolation by distance was found among the fourteen *B. decurrens* populations. Correlations between effective migrants per generation and both direct distance (r^2 = 0.0025, p = 0.522) and river km distance (r^2 = 0.0012, p = 0.600) were non-significant over all possible pairs of populations.

Tests of unidirectional gene flow due to hydrochory

Tests for non-random distribution of genetic variation

No evidence for non-random distribution of genetic variation due to unidirectional gene flow was observed in *B. decurrens*. All analyses produced non-significant regression

coefficients, regardless of the measure of allelic diversity (heterozygosity, percent polymorphic loci, or average number alleles per polymorphic locus) or model of distance from the southernmost population (river km or direct distance) tested (Table 4).

Evolutionary relationships among populations

Phenetic analysis revealed weak evolutionary relationships among populations (Figure 2). Bootstrap analysis revealed all nodes to be produced in less than 50% of trees. Tests to reconcile the observed phenogram with the hypothetical cladogram revealed the observed relationship among *B. decurrens* populations to be no different from random (mean value of randomly generated tests statistic = 59, std. dev. = 6.5; test statistic for observed phenogram = 50).

Examining the consequences of frequent local extinctions

Tests for recent population bottlenecks

Recent bottlenecks were detected in twelve of the fourteen populations tested (86%). Three populations (nos. 2, 6, and 11) displayed a marginally significant excess of heterozygosity ($p < 0.05$). Nine populations (nos. 3, 4, 5, 9, 10, 13, and 14) displayed highly significant excess of heterozygosity ($p < 0.01$).

Mating system analysis

The results of the mating system analysis performed for families from three populations indicate that *B. decurrens* is not obligate outcrossing, but is characterized by high levels. Multilocus outcrossing rates varied from 0.873 (std. dev. = 0.059) in population 10 to 0.944 (SD = 0.038) in population 1 and 0.952 (SD = 0.036) in population 9.

Tests for fine-scale population substructure

Fine-scale population structure was not detected within the two *B. decurrens* populations tested. Figure 2 shows the results of the spatial autocorrelation analysis of population 12. Results for population 5 were similar.

Examining the consequences of frequent local colonizations

Intrapopulation cluster analysis

No evidence of multiple clusters (subpopulations) was found in any of the test populations. All analyses indicated a single cluster in the sampled population (Table 5).

Admixture analysis

Simulation results revealed the Bayesian method to most accurately assign individuals to the correct source population (unpublished data). Due to the low likelihoods of each cluster analysis, and the even distribution of individuals among clusters, entire populations, rather than individual clusters, were analyzed in the admixture analysis. Multiple populations were indicated as sources of propagules for each test population (Figure 4). A small number of sources were assigned a greater proportion of individuals than expected at random, resulting in a few populations accounting for a majority of the samples in each test population. Four source populations contributed disproportionately to test population 4: nos. 2, 5, 6, and 8; four sources to test population 12: nos. 2, 5, 6, and 8; and three sources to test population 13: nos. 7, 8, and 14.

Tests for annual differences in population structure

Two of the four populations sampled in both 2000 and 2001 demonstrated significant allele frequency variation among years: for population 7, F_{ST} = 0.0297 (95% CI: 0.0148, 0.0438), and for population 9, F_{ST} = 0.0264 (95% CI: 0.0055, 0.0458). The other two populations did not demonstrate significant variation: for population 5, F_{ST} = 0.0182 (95% CI: -0.0031, 0.0424), and for population 12, F_{ST} = 0.0160 (95% CI: -0.0055, 0.0395).

Discussion

Inferred patterns of seed dispersal

Based on the reproductive biology of *B. decurrens* and the large distances separating populations, we predicted that gene flow would be primarily seed mediated and skewed in a downstream direction as a result of dispersal along the Illinois River. Previous studies of

species predominately distributed by hydrochory found limited evidence for unidirectional gene flow, as indicated by higher levels of genetic variation in downstream populations (Akimoto et al. 1998; Gornall et al. 1998; Waser et al. 1982). However, we found no significant evidence of downstream populations containing higher levels of genetic variation than upstream populations, indicating that unidirectional gene flow does not govern the genetic structure of *B. decurrens*. Two hypotheses may explain this observation. First, the Illinois River is characterized by relatively slow flow patterns, which may not be sufficient to maintain seed dispersal in a strictly unidirectional manner. In addition, severe flooding downstream may cause backwaters of 160 km (100 mi) or more to develop along the river (Kofoid 1903). As *B. decurrens* is known to establish new populations as waters recede from flooding events (Smith & Keevin 1998), it is possible that these backflows allow propagules to move in an upstream manner. Second, the potential for wildlife to act as vectors to the distribution of *B. decurrens*' seeds upstream has not been investigated. The achenes of *B. decurrens* disc flowers are characterized by two long awns, which have been shown to promote long-term flotation on still and turbid water (Smith & Keevin 1998). However, it is also likely that these awns may attach to mammals or waterfowl and be transported upstream in this manner. Given that *B. decurrens* is a prolific seed producer (ca. 50,000 achenes per plant, Smith & Moss 1998), this alternate mode of seed dispersal may be sufficient to maintain equivalent levels of genetic variation across the range of the species.

Hydrochory has also been shown to result in long-distance seed dispersal among plant populations (Akimoto et al. 1998; Kudoh & Whigham 1997). The lack of isolation by distance among populations of *B. decurrens* indicates that long-distance seed dispersal occurs with some frequency, either during population establishment or during subsequent gene flow events.

Processes influencing interpopulation genetic structure

The observed genetic differentiation between populations of *B. decurrens* is consistent with levels predicted from recurring extinction and colonization (McCauley 1991; Pannell & Charlesworth 2000; Wade & McCauley 1988; Whitlock & McCauley 1990), and with levels observed in other metapopulations (Giles & Goudet 1997; McCauley et al. 1995;

Roach et al. 2001). However, the results of our investigation reveal that the demographic processes identified *a priori* do not accurately explain the genetic structure of *Boltonia decurrens*. We predicted that near-panmictic gene flow due to seed dispersal along the river would be likely, yet the significant F_{ST} values indicate that gene flow among populations is not sufficient to curtail differentiation among populations. In addition, we found no evidence for unidirectional gene flow among populations, suggesting that the unidirectional flow of the rivers has no significant effect on the genetic structure of the metapopulation. The lack of both isolation by distance and regional structure between northern and southern populations indicates that long-distance gene flow events must occur at some frequency. Together, these data indicate that the genetic structure among *B. decurrens* populations is influenced by a variety of less obvious factors.

The most likely explanation for the observed genetic structure among populations is the process of correlated colonization. Rather than universal mixing of seed during colonization events, newly established populations are founded from propagules originating in one or a few source populations. The significant differentiation among populations (F_{ST}) is consistent with the hypothesis that propagules do not tend to mix during population establishment. Additionally, the recent genetic bottlenecks identified in the majority of sampled populations likely contribute to the significant fixation indices (see below). Although not tested directly, it is possible that these bottlenecks result from colonists originating from a small number (or single) source population. Finally, the admixture analysis was consistent with the cluster analysis in identifying a single population that contributed disproportionately to the founding of novel populations. Given the moderate differentiation among these populations and the lack of unique or diagnostic alleles, the large number of source populations identified as the origin of a small percentage of individuals is likely the result of low statistical power rather than an indication of panmictic seed migration. Based on these observations, the interpopulation genetic structure in *B. decurrens* is most likely a consequence of colonization dynamics better described by the propagule model of colonization than the migrant pool model.

Processes influencing intrapopulation genetic structure

Although unidirectional gene flow was not found to significantly affect the genetic structure of *B. decurrens*, our analyses have revealed other processes that influence the distribution of allelic variation within and among populations. The mating system analysis indicates that *B. decurrens* is capable of higher rates of self-fertilization than was indicated by prior studies (hand pollination experiments produced 5% viable seed; (Tofari 2000). This mixed mating system appears to be variable among populations, with multilocus outcrossing rate estimates ranging from 0.87 to 0.95. In addition, the correlation of paternity among outcrossed progeny indicates that the number of effective pollen donors per maternal plant is low (mean 1.8), suggesting that a small number of pollen donors contribute disproportionably to the seed set on each mother plant. Given that *B. decurrens* is apparently capable of self-fertilization and individual plants may receive pollen from relatively few donors, are these processes sufficient to explain the large fixation indices observed within populations (mean F_{IS} = 0.192, range 0.118-0.345)? In equilibrium populations, the expected relationship between the mating system and the fixation index (F) is F = (1-t)/(1+t), where t is the outcrossing rate (Clegg 1980). Substituting t_m = 0.87 for t, mixed mating in *B. decurrens* along could account for fixation indices as great as F = 0.07. This suggests that the mating system alone cannot explain the observed levels of fixation in *B. decurrens*. Several other observations also indicate that the mating system is not solely responsible responsible for the observed excess of homozygosity. First, given high rates of population turnover in *B. decurrens*, the fixation of individual populations may be unlikely to reach this equilibrium value. Second, for highly outcrossing populations, fixation is expected to be low, yet for population 1, t_m = 0.944 while F_{IS} = 0.154 and is significantly greater than zero (Table 2). Third, given the lack of detectable fine-scale genetic structure within populations (Figure 2), biparental inbreeding is unlikely, even if mating is largely restricted to a few neighboring plants. As a result, factors other than the mating system and biparental inbreeding must be operating to explain the significant fixation indices characteristic of *B. decurrens* populations.

In addition to inbreeding *per se*, the pooling of multiple subpopulations into a single sample can result in an apparent excess of homozygotes within a population (i.e., a Wahlund

effect, (Hartl & Clark 1997). In *B. decurrens*, the process of population establishment (colonization) may occur with propagules drawn from a small number of source populations. The significant differentiation observed among populations of *B. decurrens* ($F_{ST} = 0.098$) indicates that populations are not established with founders drawn at random from the metapopulation as a whole. Under the propagule pool model, F_{IS} for individual populations could be comparable to F_{ST} for the total population as a result of the Wahlund effect. Thus, this model of dispersal and colonization, like the mating system, can account for some, but not all, of the fixation observed within individual populations.

Our analyses also indicate that a high proportion of populations (86%) have recently undergone a genetic bottleneck. While we cannot determine whether bottlenecks tend to occur during or subsequent to population establishment, previous field studies (Smith 1994) indicate that some new populations are established by large numbers of individuals (tens of thousands). These observations imply that bottlenecks are not always the result of a limited number of founders, but may also occur after population establishment, as population numbers begin to decline. Together, these data indicate that the observed fixation indices are most likely the result of a combination of processes including the mating system, the incomplete mixing of seed at population establishment, and genetic bottlenecks at population founding and senescence.

Implications for conservation

The conclusions drawn from this study should aid in efforts to conserve *Boltonia decurrens*, as well as other threatened floodplain species displaying similar life histories (e.g., *Aster kantoensis*, Takenaka et al. 1996) and seed dispersal biology (e.g., *Hibiscus moscheutos*, Kudoh & Whigham 1997). First, the genetic structure of this species is consistent with that expected of a metapopulation, and confirms the significance of processes such as local extinction and colonization in the life history of the species. This process of regular population turnover emphasizes the importance of available unoccupied patches to the species survival (Hanski 1998), especially as appropriate habitat becomes increasingly fragmented. Second, our analyses failed to identify unique (or private) alleles within populations and found no indication of local ecotypes characteristic of different regions of

this species' range. These results indicate that *B. decurrens* is currently functioning as a singly evolutionary significant unity (ESU) and that management protocols should address it accordingly. Third, the absence of private alleles and local ecotypes, coupled with the lack of isolation by distance, indicate that efforts to reestablish populations of *B. decurrens* could proceed using seed from a small number of populations, and that collections need not be restricted to neighboring populations. Fourth, while we cannot conclude that our data represent historical levels of variation within and among populations of *B. decurrens*, it can be used as a baseline against which to monitor future levels and geographic patterns of genetic structure. Finally, the lack of isolation by distance among populations emphasizes the importance of stochastic severe flooding events sufficient to distribute seed across the range of this species, and indicates that the restoration of historic flooding patterns to the Illinois River floodplain would provide the highest likelihood of long-term persistence of this threatened species.

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Table 1. Names, locations and sample sizes for thirteen populations of *Boltonia decurrens* and one population of *B. asteroides*. Population numbers represent the location of the *B. decurrens* populations along the Illinois River, from upstream to downstream. N = sample size used to estimate allele frequencies for each population. Year = year that population was first reported. See text for sample sizes of analyses listed below. See Figure 1 for the relative location of each population.

No.	Name	Location	Coordinates	N	Year
B. decurrens					
1°*	Hennepin Bridge	Bureau Co., IL	41° 15' N, 89° 21' W	96	1984
2	Sparland	Marshall Co., IL	40° 56' N, 89° 28' W	48	1984
3	Woodford County Conservation Area	Woodford Co., IL	40° 53' N, 89° 27' W	16	1984
4°	McClugage Bridge	Peoria Co., IL	40° 43' N, 89° 33' W	96	1989
5^‡	Rice Lake	Fulton Co., IL	40° 30' N, 89° 54' W	48	1984
6	Havana	Mason Co., IL	40° 18' N, 90° 04' W	30	1984
7‡	Anderson Lake	Fulton Co., IL	40° 12' N, 90° 12' W	48	1984
8	Sanganois State Fish & Wildlife Area	Schuyler Co., IL	39° 00' N, 90° 33' W	48	1984
9*‡	Meredosia Lake	Morgan Co., IL	39° 52' N, 90° 33' W	48	1984
10*	Gilbert Lake	Jersey Co., IL	38° 58' N, 90° 30' W	48	1984
11	West Alton	St. Charles Co., MO	38° 58' N, 90° 30' W	48	1984
12°‡	Horseshoe Lake	Madison Co., IL	38° 41' N, 90° 06' W	96	1994
13°^	Waste Management	Madison Co., IL	38° 40' N, 90° 07' W	96	1994
14	Fairmont City	St. Clair Co., IL	38° 39' N, 90° 07' W	48	1984
B. asteroides					
BA	Stump Lake (SL)	Calhoun Co., IL	39° 00' N, 90° 33' W	48	na

° denotes populations sampled for admixture analysis

* denotes populations included in the mating system analysis

^ denotes populations sampled for spatial autocorrelation analysis

‡ denotes populations sampled in both 2000 and 2001 to test for annual differences in genetic structure

Table 2. Summary genetic statistics for 14 allozyme loci evaluated for 14 populations of *Boltonia decurrens* across the range of the species. A = average number of alleles per locus, A_p = average number of alleles per polymorphic locus, P = percent polymorphic loci, H_e = expected heterozygosity at Hardy-Weinberg equilibrium, F = population fixation index with standard errors in parentheses.

Population	A	A_p	P	H_e	F
1	2.308	2.546	84.62	0.351	0.154 (0.058)
2	2.385	2.800	76.92	0.309	0.105 (0.061)
3	2.077	2.750	61.94	0.333	0.116 (0.070)
4	2.231	2.600	76.92	0.312	0.081 (0.031)
5	2.385	3.000	69.23	0.332	0.101 (0.049)
6	2.167	2.750	66.67	0.276	0.044 (0.065)
7	2.154	2.875	61.45	0.258	0.082 (0.047)
8	2.385	2.800	76.92	0.297	0.208 (0.054)
9	2.385	2.800	76.92	0.371	0.074 (0.062)
10	2.308	2.889	69.23	0.352	0.157 (0.058)
11	2.308	2.700	76.92	0.295	0.115 (0.059)
12	2.385	2.800	76.92	0.211	0.142 (0.065)
13	2.462	2.900	76.92	0.330	0.175 (0.052)
14	2.462	2.900	76.92	0.322	0.086 (0.048)

Table 3. Analysis of hierarchical genetic differentiation among populations of *Boltonia decurrens*. The two-level hierarchy includes all populations included in a single metapopulation with no regional substructure. The three-level hierarchy divides the populations into northern and southern regions. F_{IS} is the fixation index within populations, F_{ST} estimates allele frequency variation among populations relative to the total metapopulation, F_{RT} estimates allele frequency variation among regions. 95% confidence intervals are given in parentheses.

	Two-level hierarchy	Three-level hierarchy
F_{IS}	0.192 (0.096, 0.282)	0.192 (0.096, 0.282)
F_{ST}	0.098 (0.080, 0.114)	n/a
F_{SR}	n/a	0.104 (0.081, 0.125)
F_{RT}	n/a	0.013 (-0.003, 0.034)

n/a = not applicable

Table 4. Correlation coefficients and associated tests of significance for all tests of anisotropic gene flow among populations of *Boltonia decurrens*. Latitude was measured on site using a hand-held Global Positioning System device. River km estimates the river distance upstream from the southernmost population. A_p is the mean alleles per polymorphic locus, P is the percent polymorphic loci, H_o is the observed heterozygosity, and H_e is the equilibrium heterozygosity. See text for details.

Measure of Allelic Diversity	Latitude	River km
A_p	$R^2 = 0.199$; $p = 0.110$	$R^2 = 0.256$; $p = 0.065$
P	$R^2 = 0.016$; $p = 0.669$	$R^2 = 0.004$; $p = 0.829$
H_o	$R^2 = 0.093$; $p = 0.290$	$R^2 = 0.102$; $p = 0.265$
H_e	$R^2 = 0.048$; $p = 0.451$	$R^2 = 0.059$; $p = 0.404$

Table 5. Testing for subpopulation structure as evidence for admixture upon population establishment in *B. decurrens*. Columns represent estimated posterior probabilities of *k*, the number of clusters present in three test populations, nos. 4, 12, and 13.

k	Pop. 4	Pop. 12	Pop. 13
1	1	1	1
2	~ 0	~ 0	~ 0
3	~ 0	~ 0	~ 0
4	~ 0	~ 0	~ 0
5	~ 0	~ 0	~ 0
6	~ 0	~ 0	~ 0
7	~ 0	~ 0	~ 0
8	~ 0	~ 0	~ 0

Table 6. Relative contribution of propagules by source populations to colonization events as revealed by admixture analyses. Values indicate the proportion of individuals assigned to the source population from a total of 96 sampled at each test population.

Source Population	Pop. 4	Pop. 12	Pop. 13
1	0.12	0.00	0.02
2	0.12	0.18	0.01
3	0.08	0.00	0.08
4	N/A	0.04	0.02
5	0.17	0.13	0.01
6	0.19	0.35	0.04
7	0.03	0.05	0.16
8	0.16	0.13	0.11
9	0.02	0.02	0.02
10	0.05	0.00	0.02
11	0.04	0.06	0.07
14	0.02	0.02	0.43

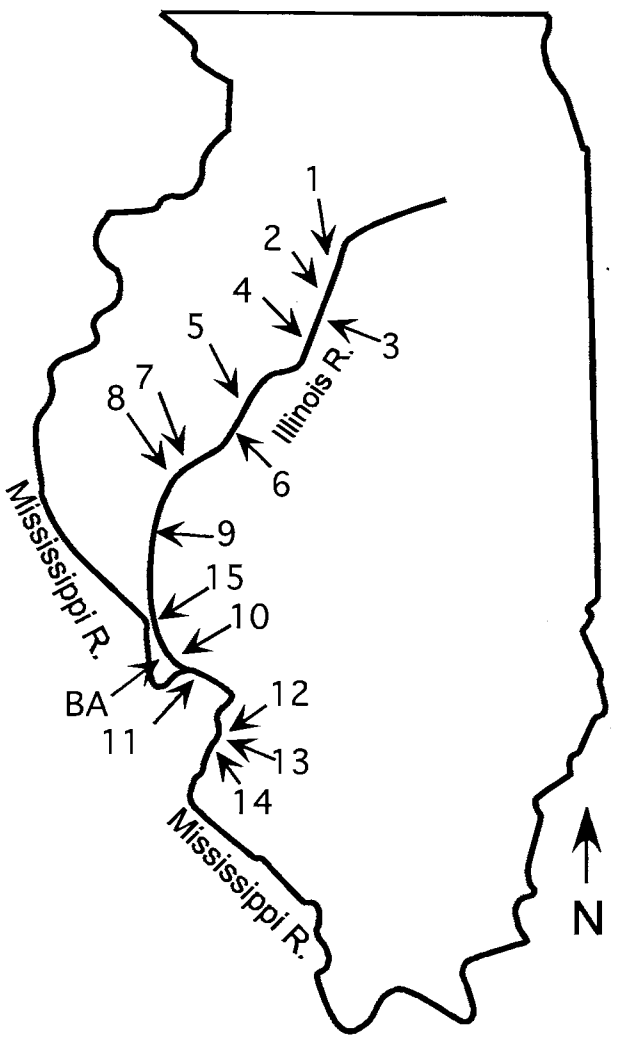

Figure 1. Locations of 14 populations of *Boltonia decurrens* sampled for this study. Although these collections do not constitute an exhaustive search for *B. decurrens*, the study sites do encompass the natural range of the species. See Table 1 for details.

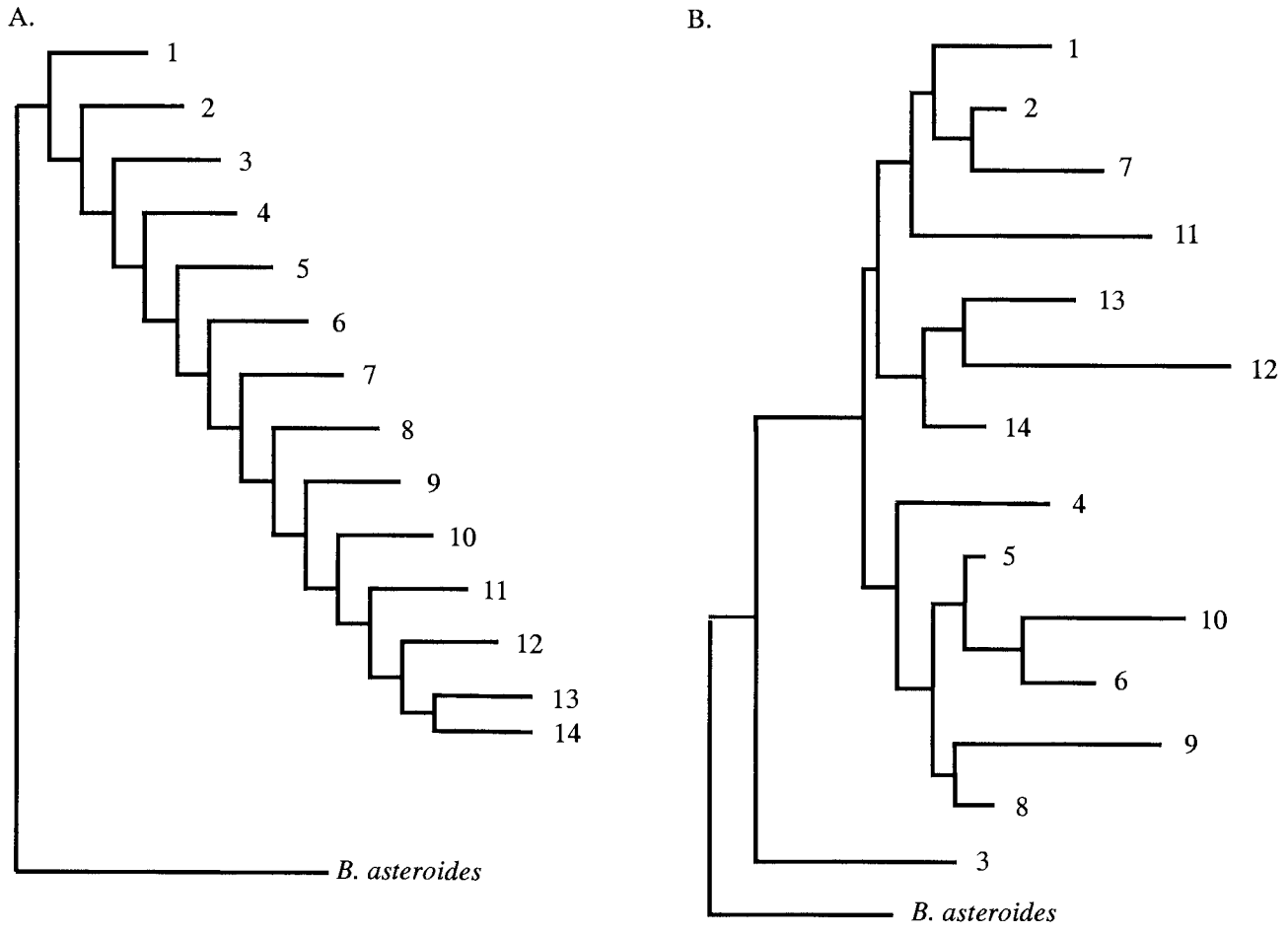

Figure 2. Hypothetical and observed population phenograms for fourteen populations of *Boltonia decurrens.* A) Hypothetical phenogram expected as a consequence of unidirectional gene flow due to hydrochory. B) Population phenogram based on allozyme data constructed from Nei's genetic distances using neighbor-joining methods. All nodes were produced in less than half of 1000 bootstrap analyses.

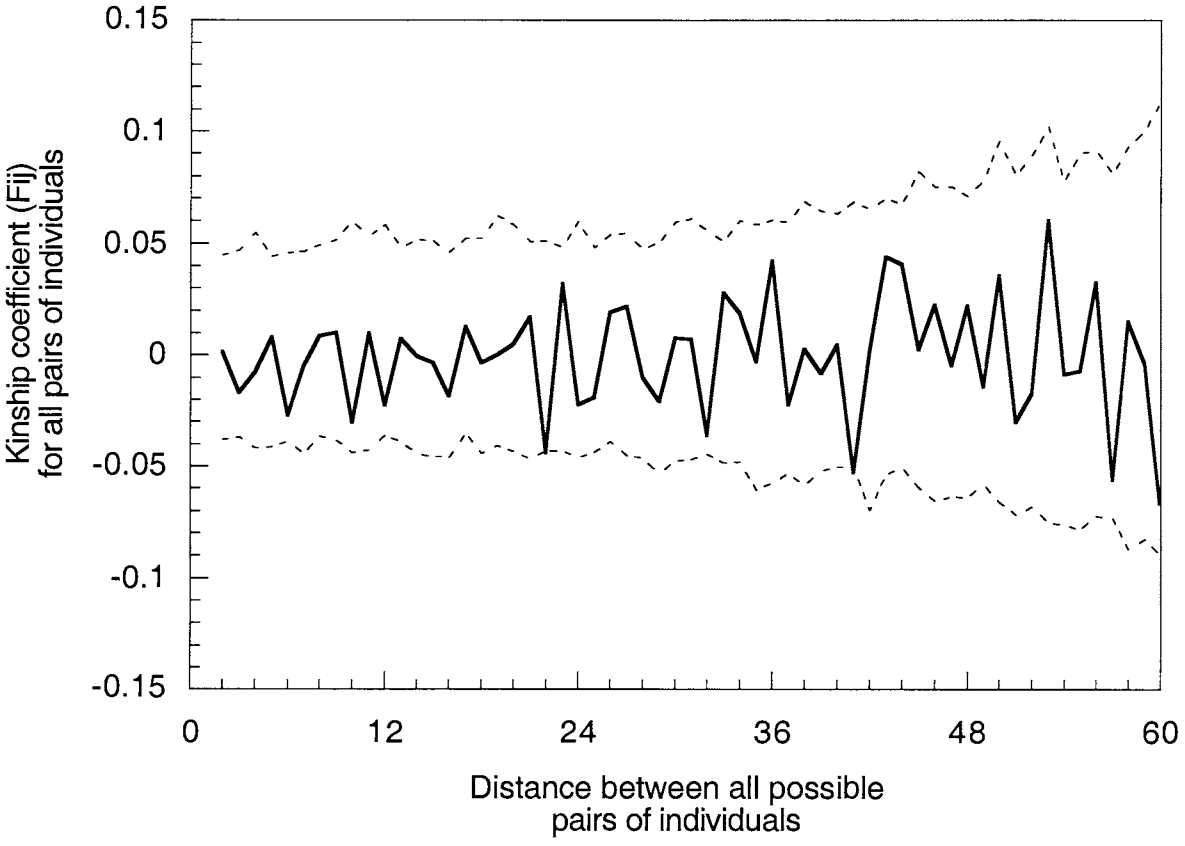

Figure 3. Observed correlation of relatedness and distance for all possible pairs of individuals sampled on a transect at population 12. Solid line indicates estimates of kinship coefficients associated with all possible pairs of individuals collected at 1 m intervals along a transect. Dashed lines indicate the 95% confidence interval around the null hypothesis of no correlation between distance and kinship, as estimated by bootstrap analysis.

Appendix 1. Allele frequencies for thirteen allozyme loci estimated for fourteen populations of *Boltonia decurrens*.

Locus	HP	S	MC	WC	RL	AL	SA	ML	WA	GL	WM	HL	FC	HA
PGI														
4	0.8177	0.9167	0.9531	1.0000	0.8750	1.0000	0.9688	0.9479	1.0000	0.6354	0.9789	0.9948	0.9792	0.8659
6	0.1823	0.0833	0.0469		0.1250		0.0312	0.0521		0.3646	0.0211	0.0052	0.0208	0.1341
SKDH														
2	0.6198	0.4674	0.6146	0.6765	0.2766	0.4787	0.4062	0.3085	0.5729	0.3830	0.4205	0.4894	0.3511	0.4000
3	0.3802	0.4674	0.3854	0.3235	0.6809	0.2979	0.5104	0.6383	0.3438	0.5957	0.1364	0.5000	0.2872	0.6000
4									0.0104	0.0213				
5		0.0543			0.0426	0.2234	0.0625		0.0729		0.3920	0.0106	0.3085	
6		0.0109					0.0208	0.0532			0.0511		0.0532	
PGD2														
4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
PGD3														
2	0.1927	0.5208	0.3906	0.3333	0.5521	0.5213	0.6170	0.4787	0.1296	0.4149	0.6941	0.8105	0.5233	0.4762
4	0.5000	0.1979	0.1823	0.1944	0.1875	0.4468	0.0851	0.0957	0.4444	0.2234	0.2118	0.0789	0.1047	0.2143
6	0.3073	0.2812	0.4271	0.4722	0.2604	0.0319	0.2979	0.4255	0.4259	0.3617	0.0941	0.1105	0.3721	0.3095
ME														
2	0.3229	0.4271	0.3830	0.5000	0.3958	0.3125	0.1771	0.1979	0.2812	0.2717	0.3617	0.6947	0.5000	0.2679
3	0.5469	0.3958	0.6011	0.3056	0.4896	0.6042	0.7292	0.4062	0.7188	0.7283	0.5745	0.2263	0.4894	0.5893
4	0.1302	0.1771	0.0160	0.1944	0.1146	0.0833	0.0938	0.3958			0.0638	0.0789	0.0106	0.1429
TPI1														
4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
TPI2														
2	0.2609	0.0957	0.3906	0.1389	0.3690	0.1042	0.5109	0.4792	0.3372	0.4889	0.3316	0.6413	0.2396	0.7125
4	0.4348	0.7128	0.1823	0.7778	0.4167	0.7396	0.3696	0.4271	0.6395	0.4222	0.3947	0.3261	0.5938	0.1625
6	0.3043	0.1915	0.4271	0.0833	0.2024	0.1562	0.1196	0.0104	0.0233	0.0444	0.1474	0.0326	0.0729	0.1125
8					0.0119			0.0833		0.0444	0.1263		0.0938	0.0125

Appendix 1 continued.

Locus	HP	S	MC	WC	RL	AL	SA	ML	WA	GL	WM	HL	FC	HA
FE														
2	0.3462	0.1556	0.0659	0.4444	0.1458	0.4130	0.1889	0.3854	0.0625	0.0682	0.1702	0.0684	0.1702	0.1375
4	0.4121	0.4667	0.3187	0.3056	0.4062	0.4239	0.5333	0.3438	0.8333	0.4318	0.6968	0.8474	0.4574	0.5250
6	0.2418	0.3778	0.6154	0.2500	0.4062	0.1630	0.2778	0.2708	0.1042	0.4773	0.1330	0.0789	0.3723	0.3375
8					0.0417					0.0227		0.0053		
MNR														
2	0.8698	0.9167	0.6316	0.4706	0.7708	0.9062	0.6087	0.5729	0.6739	0.7955	0.6611	0.9521	0.7812	0.9600
6	0.1302	0.0833	0.3684	0.5294	0.2292	0.0938	0.3913	0.4271	0.3261	0.2045	0.3389	0.0479	0.2188	0.0400
DIA														
2	0.0260	0.0625	0.0805	0.0882	0.1042	0.0625	0.0952	0.0938	0.0426	0.0227	0.0056	0.0337	0.1042	
4	0.7917	0.6458	0.7356	0.5294	0.6875	0.8750	0.9688	0.7396	0.5638	0.6023	0.7247	0.8652	0.6979	
6	0.1823	0.2917	0.1839	0.3824	0.2083	0.0625	0.0833	0.1667	0.3936	0.3750	0.2697	0.1011	0.1979	
UGPP														
2							0.0312					0.0417	0.0312	
4	0.8526	0.9583	0.9844	1.0000	1.0000	1.0000	0.9688	0.7500	0.9896	1.0000	1.0000	0.9583	0.9688	1.0000
6	0.1474	0.0417	0.0156					0.2500	0.0104					
IDH														
2	0.6989	0.8409	0.8389	0.5500	0.8021	0.8723	0.8587	0.6667	0.8958	0.4889	0.6860	0.8407	0.8286	0.8333
4	0.1818	0.1250	0.1278	0.1000	0.1458	0.0106	0.0109	0.2444	0.0729	0.1111	0.1279	0.1429	0.1143	0.0278
6	0.1193	0.0341	0.0333	0.3500	0.0521	0.1170	0.1304	0.0889	0.0312	0.4000	0.1860	0.0165	0.0571	0.1389
PGM														
2	0.0054								0.2128		0.0549			
4	0.9946	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.7872	1.0000	0.9451	1.0000	1.0000	1.0000

CHAPTER 3. EVIDENCE OF LOW-FREQUENCY HYBRIDIZATION BETWEEN *Boltonia decurrens*, A FEDERALLY THREATENED HERB, AND *B. asteroides*, A WIDESPREAD CONGENER

A paper prepared for submission to *American Journal of Botany*

J. A. DeWoody, J. D. Nason and M. Smith

Abstract

Boltonia decurrens is a federally threatened herb endemic to the Illinois River floodplain, which is currently restricted to fewer than 25 populations. We tested for hybridization between this species and a widespread congener, *B. asteroides*, at the only two sites where they are known to occur in sympatry. Analyses of the sympatric sites failed to identify individuals with intermediate, hybrid-like phenotypes. Similarly, genetic analyses (likelihood based methods using allozyme data) failed to detect the presence of F1 hybrids in either sympatric population, but did reveal low frequencies of F2 and backcross progeny. Principle components analysis identified six characters, two morphological and four genetic, that together explain 43% of the observed variation. Although more powerful molecular markers are necessary to detail the pattern and extent of introgression, these results indicate that interspecific hybridization does occur in mixed stands of *B. decurrens* and *B. asteroides*, and that morphological analysis alone is not sufficient to detect hybrid individuals. Given the limited sympatry of *B. decurrens* with its congeners and the restricted interspecific recombination described here, we conclude that hybridization does not pose an immediate threat to the genetic integrity of this species or its current threatened status under the U.S. Endangered Species Act.

Introduction

Interspecific hybridization is a serious concern for the fitness and protection of threatened and endangered species. The hybridization of a rare species with a common one

may lead to decreased fertility, genetic swamping, and ecological competition with hybrids that can contribute to the further decline of the rare taxon (Levin, Francisco-Ortega, and Jansen, 1996; Rhymer & Simberloff, 1996). Rare species shown to hybridize with common congeners were not originally protected under the U.S. Endangered Species Act (U.S. ESA) (Soltis & Gitzendanner, 1999). Current interpretation of the U.S. ESA by the U.S. Fish & Wildlife Service, however, allows the listing of threatened taxa if hybrid progeny more closely resemble the listed parent than the intermediate of the two parental species (Rhymer & Simberloff, 1996; Whitham & Maschinski, 1996; Soltis & Gitzendanner, 1999). Since extensive hybridization with a common taxon may not only impact the population fitness, but also void a species' protected status, it may be necessary to design management strategies that minimize the potential for hybridization and introgression to take place. Where rare and common congeners occur in sympatry, a clear understanding of the extent and potential for hybridization and introgression is necessary both to guide public policy and to develop short and long-term plans for species conservation.

From the perspective of managing rare species, the extent of interspecific hybridization can be classified on a gradient between two extremes. At one extreme, hybridization may be limited to scattered F1 individuals (Nason, Ellstrand, and Arnold,1992 and sources therein) or to a stable hybrid zone (Howard et al., 1997), in which the introgression of genes between species is low. In these cases, a threatened species may maintain its genetic integrity and qualify or maintain its protected status.

At the other extreme, interspecific hybridization may be both extensive and asymmetrical, potentially resulting in the genetic assimilation of the rare into the more common species, since the likelihood of an interspecific mating event is inversely correlated to the species' proportion at the site (Levin, Francisco-Ortega, and Jansen, 1996). The genetic assimilation of a rare species by a common species has been identified as one mechanism leading to the decline of rare species, e.g., *Cercocarpus traskiae* (Rieseberg et al., 1989). In these cases, the listing or continued protected status of the rare species is not ensured, and substantial management efforts may be required to maintain the genetic integrity of the species by minimizing the potential for introgression.

Because the policy protecting rare and endangered species depends on the hybridization status, it is necessary to quantify the occurrence and extent of hybridization in natural populations. If the two parental species are well differentiated, hybrid individuals may be easily identified by intermediate morphologies and recombinant genotypes at genetic marker loci (Bleeker & Hurka, 2001; Nason, Ellstrand, and Arnold, 1992). However, available morphological and genetic data may not always permit the unambiguous identification of hybrids. For example, the recognition of hybrids in natural populations may be complicated by dominance effects or the expression of extreme, transgressive phenotypes (Schwarzback, Donovan, and Rieseberg, 2001). Further, for closely related species, morphological differentiation may simply not be sufficient to permit the recognition of hybrid individuals from either parental taxa. Similarly, in the absence of diagnostic loci or species-specific alleles, parental species will produce overlapping sets of multilocus genotypes, limiting the power of the genetic data to discriminate hybrids. As rare taxa may often be closely related to widespread congeners, combining morphological and genetic approaches may provide the most conclusive evidence of hybridization and introgression (Bleeker & Hurka, 2001, Bartish, Rumpunen, and Nybom, 2000).

Boltonia decurrens (T&G) Wood (Asteraceae) is a federally threatened floodplain endemic imperiled by habitat destruction resulting from flood control measures, the conversion of floodplain habitat to row-crop agriculture, and urban development along the Illinois River (Bellrose et al., 1983; Schwegman & Nyboer, 1985). *B. asteroides* (L.) L'Hér., in contrast, is a widely distributed, facultative floodplain species, occurring across most of the eastern United States (Gleason & Cronquist, 1991). Previous observations have failed to detect intermediate morphologies indicative of hybridization between these species (Schwegman & Nyboer, 1985). Hand-pollination experiments, however, have shown that viable seed is produced when *B. asteroides* pollen is crossed onto *B. decurrens* (Tofari, 2000). The two species are known to be sympatric in two locations near Beardstown and Frederick, IL. Although previously present in approximately equal proportions at the Beardstown location, recent censuses indicate a decline of the *B. decurrens* and an expansion of the *B. asteroides* populations. The Frederick site was long considered to contain only *B. decurrens* until the first observation of a *B. asteroides* individual in 2000 (P. Mettler,

Southern Illinois University, personal communication). Given the past and current compositions of the populations at the two sympatric sites, we predict that patterns of hybridization will differ between the sites: Beardstown is expected to contain a larger population of *B. asteroides*, with *B. asteroides* potentially swamping the dwindling *B. decurrens* population, while Frederick is expected to be dominated by the *B. decurrens* population, which may be swamping the colonizing *B. asteroides* individuals.

Here we report the use of morphological and molecular techniques to test the hypothesis that *B. decurrens* is hybridizing with the more common and widespread *B. asteroides* where the two species co-occur. First, we ask whether morphological differences between putatively pure parental species populations are sufficient to permit the identification of hybrid individuals. We assess a number of morphological characters, identify those that exhibit significant differentiation between species, and determine the power of each to discriminate between species. Second, we characterize allozyme allele frequencies for each species and use likelihood methods to determine the power of these markers to distinguish parental species, as well as first- and second-generation products of hybridization between them. Third, we use the diagnostic morphological characteristics and allozyme markers, both separately and jointly, to estimate the frequency of hybridization and to identify hybrid individuals in the two sympatric populations. Finally, we discuss the occurrence, extent, and asymmetry of hybridization between these species, and its implications for the management and preservation of *B. decurrens* populations, as well as its classification under the U.S. ESA.

Materials and Methods

Study species and sites

As an early successional floodplain specialist, *B. decurrens* colonizes newly disturbed habitat following flooding events. Seeds require light and saturated, sandy soil to germinate (Smith & Keevin, 1998), and once established, individuals are shade-intolerant, typically resulting in extinction of populations as later-successional species out-compete *B. decurrens*

for light (Smith, Wu, and Green, 1993). Seedlings produce rosettes that bolt in the spring (either the same year as germination or the following season) to produce flowering stems that can be over 2 m tall in the late summer and early fall. Flowering stems produce basal rosettes in the fall, which remain green over winter and bolt the following spring. Successive establishment of basal rosettes around a mother stem eventually leads to the death of the genet due to crowding and competition for resources (Smith, personal observation). Cauline leaves are decurrent, and produce scars on stems after senescence in the fall. Flowering heads (capitula) are radiate, with pistillate ray flowers producing three-sided achenes, and perfect disc flowers producing flat achenes with long awns that aid in water dispersal (Smith & Keevin, 1998). *B. decurrens* is a prolific seed producer, averaging 50,000 achenes per plant (Smith & Moss, 1998).

Boltonia asteroides contrasts with *B. decurrens* as a widespread, facultative floodplain species that is not necessarily out-competed for light and water by later successional species. While its morphology and life history are similar to those of *B. decurrens*, the most apparent physical difference between the species is the lack of decurrent leaves and the production of rhizomes in *B. asteroides*. Also, Morgan (1966) reported that flower number per capitulum differs significantly between the two species.

Morphological data collection

Between September 15 and September 18 and again between October 27 and October 29, 2001, morphological data were collected for individual plants at two allopatric and two sympatric sites. The two allopatric populations were sampled in order to characterize putatively pure *B. decurrens* and *B. asteroides* individuals. Twenty-four individuals each were sampled at random from the pure *B. decurrens* population at Gilbert Lake (GL) and the pure *B. asteroides* population at Stump Lake (SL) (see Table 1 and Fig. 1). To test for intermediate morphologies indicative of hybrid individuals, we sampled 47 individuals each from Beardstown (BT) and Frederick (F), the only two sites where these species are known to be sympatric (see Table 1 and Fig. 1). Plants were sampled at random at F, where *B. asteroides* individuals have only recently been observed scattered within the *B. decurrens*

population. At BT, plants were sampled along a transect crossing from an area of *B. decurrens*-like plants, through the putative hybrid zone, and into a large stand of *B. asteroides*-like plants. While the *B. decurrens* population at Beardstown was once similar in size to the *B. asteroides* population (Smith, personal observation), successive dry summers have favored the growth of *B. asteroides* over *B. decurrens* in recent years.

The following morphological measurements were made in the field on each sampled plant : ratio of internode length to decurrence length for the first two internodes below the first true branch (defined as the first branch to successfully produce an inflorescence), leaf length and width for two leaves located at branching nodes, leaf margin (entire, toothed or very toothed), and leaf texture (glaucous, intermediate, or tough). Although the presence of basal rosettes in *B. decurrens* is taxonomically recognized as a defining feature of the species, the protected status of the species, as well as one study site occurring on an archaeological preserve, prevents the destructive sampling necessary to positively identify basal rosettes from rhizomes. As a result, this trait is not a viable option for monitoring efforts and was omitted from our study.

For each plant we also collected and preserved (in 70% ethanol) three flower heads (capitula) for later morphological analysis. One head per plant was dissected to determine the number of disc flowers, number of ray flowers, maximum capitulum width, and maximum capitulum height (defined as the distance from the point of phyllary insertion to the tallest point of the capitulum when all flowers are removed). In addition, the length of 5 disc flowers was measured to determine the average disc flower length per plant. In total, ten morphological traits were measured or characterized per plant.

Morphological data analysis

In order to determine whether morphology differs between *B. decurrens* and *B. asteroides* sampled from the two pure allopatric populations (*B. decurrens* at GL, *B. asteroides* at SL), we conducted a univariate analysis of variance for each morphological character studied. We tested for equal variance between populations using O'Brien's test of equal variance, and used Welch's test of equal means for those characteristics with unequal

variances. All morphological statistical tests were completed using JMP version 4.0.4 (SAS Institute, 2001). In order to identify those characters providing the greatest resolution between species (traits of greatest use in future monitoring efforts), we conducted a power analysis for all morphological characteristics that differed significantly between species. Those characteristics providing the highest power to differentiate between species will be the most effective in monitoring efforts.

Allozyme analysis

For each plant sampled for morphological characteristics, leaf tissue was frozen in liquid nitrogen and stored at –80° C until protein extraction. Tissue was ground in 0.5 mL of the phosphate extraction buffer of Soltis et al. (1983) modified to contain 6% PVP. The resulting protein mixture was absorbed onto 3 mm X 7 mm Whatman brand (Maidstone, England) chromatography paper wicks and stored at –80° C until electrophoresis.

Allozymes were separated by electrophoresis in 10% starch horizontal gels made with three buffer systems: morpholine citrate at pH 6.1 (MC6.1) and pH 7.2 (MC7.2, from Murphy et al., 1996), and lithium hydroxide-borate "System 8" (S8, modified from Soltis et al., 1983). Samples were assayed for 10 protein stains, resulting in 12 putative loci. Seven loci were resolved in MC6.1: malic enzyme (ME1, EC 1.1.1.40), 6-phosphogluconate dehydrogenase (PGD2 and PGD3, EC 1.1.1.44), glucose-6-phosphate isomerase (GPI1, EC 5.3.1.9), shikimic dehydrogenase (SKDH1, EC 1.1.1.25), and triose-phosphate isomerase (TPI1 and TPI2, EC 5.3.1.1). Three loci were resolved in MC7.2: isocitrate dehydrogenase (IDH1, EC 1.1.1.42), phosphoglucomutase (PGM1, EC 5.4.2.2) and UTP-glucose-1-phosphate uridylyltransferase (UGPP1, EC 2.7.7.9). Two loci were resolved in S8: dihydrolipoamide dehydrogenase (DDH1, EC 1.8.1.4), and fluorescent esterase (FE1, EC 3.1.1.-). All stain recipes were adapted from (Wendel & Weeden, 1989) except UGPP (Manchenko, 1994). All isozyme loci produced banding patterns consistent with published protein structures and diploid, Mendelian inheritance.

In addition to the leaf tissue collected for the morphological analyses described above, 24 additional plants were sampled at random from the pure *B. decurrens* and *B.*

asteroides populations (GL and SL, 48 individuals total) in order to obtain more robust allele frequency estimates for the two species.

Genetic data analyses

Quantification of genetic variation within populations

We quantified genetic variation within the two species to determine if variability exists between *B. decurrens* and *B. asteroides*. In order to quantify allelic variation within each species, we estimated standard measures of genetic diversity for each pure parental population: percent polymorphic loci (P), mean number of alleles per locus (A), mean number of alleles per polymorphic locus (A_p) and the expected heterozygosity under Hardy-Weinberg equilibrium (H_e).

Population-level estimates of contemporary hybridization

Population-level tests for contemporary hybridization were conducted for both pure parental populations as well as the two putative hybrid populations using the likelihood methods of Nason, Heard, and Williams (in press). Given sufficient allele frequency variation among species, this approach can distinguish between pure populations of either species, mixed stands of pure species, or hybrid swarms. The method assumes hybridization is limited to the first two generations of recombination between species, thus constraining estimates to six potential genealogical classes: pure *B. decurrens* (P1), pure *B. asteroides* (P2), first and second generation hybrids (F1, F2) and first generation backcrosses to the first (BP1) and second (BP2) parental species. We expect the frequencies of each genealogical class to be consistent with the recent field observations at each sympatric site. That is, at BT where *B. decurrens* appears to be swamped by *B. asteroides*, we expect to observe high frequencies of P2 with lower frequencies of P1 and BP2. Similarly, at F, where the *B. decurrens* population may be swamping *B. asteroides* individuals, we expect to observe high frequencies of P1 with low frequencies of P2 and BP1.

As described in Nason, Heard, and Williams (in press), Bayesian likelihood methods are used to estimate the genealogical class frequencies present in a putative hybrid population given the observed sample multilocus genotypes and allele frequencies for each parental source population. The method assumes equal prior probabilities, and estimates the likelihood of an individual belonging to each genealogical class based on its observed multilocus genotype. Probabilities are summed over individuals in the sample population to obtain posterior estimates of the genealogical class frequencies. The priors are equated to the posterior values and the process iterated until estimates of the relative frequencies of each genealogical class within the population converge.

We also tested for evidence of hybridization at the population level using the method of Rieseberg, Baird, and Desrochers (1998). Rather than estimating the relative frequencies of each genealogical class, this method estimates the proportional input of two parental species to a hybridized population. Like Nason, Heard, and Williams (in press), this method relies on a sample of genotypes from the hybridized population and estimates of allele frequencies from pure parental species source populations. This method differs however, in that estimates are calculated for individual alleles and then averaged over loci, as opposed to being calculated for intact, multilocus genotypes.

Identification of hybrid individuals

In order to identify hybrid individuals in both sympatric sites we conducted individual assignment tests as described in Nason, Heard, and Williams (in press). For each individual sampled, we calculated the log-likelihood of its assignment to each of the six genealogical classes (see above) based on its multilocus genotype and allele frequencies in the parental species. These values were first used to conduct a likelihood ratio test of the null hypothesis that the individual belonged to the *B. decurrens* parental genealogical class. Individuals rejected as P1 were further analyzed to assign them to one of the remaining genealogical classes, in the order: P2, F1, F2, BP1, or BP2. The null distribution of the likelihood ratio test statistic was constructed via Monte Carlo simulations of 10000 individuals with the

critical value for each individual test adjusted to achieve an experiment-wise error rate of α=0.05 using the Dunn-Sidak method (Sokal & Rohlf, 1995).

We estimated the statistical power of our genetic analyses to differentiate between genealogical classes by simulating 10000 multilocus genotypes (individuals) for each class based on the parental species' allele frequencies. As described in Nason, Heard, and Williams (in press), we used the distribution of the likelihood ratio test statistic calculated for the simulated individuals to estimate the power to correctly reject the null hypothesis of being P1 in origin for P2, F1, F2, and backcross (BP1 and BP2) individuals.

Combined morphological and genetic analyses

Principle components analysis

In order to determine which character(s) from our complete analysis best explain the variation between *B. decurrens* and *B. asteroides*, and would thus be most useful for monitoring efforts, we conducted a principle components analysis of the combined data set of the ten morphological characteristics plus the independent probabilities of assignment to each of the six genealogical classes (P1, P2, F1, F2, BP1, BP2) based on genetic data. The characters whose eigenvectors contributed the greatest relative absolute magnitude to the first two principle components were determined to be highly descriptive. All analyses were completed via principle components analysis of the correlation matrix under the Multivariate platform in JMP version 4.0.4 (SAS Institute, 2001).

Bivariate analysis

In order to maximize our ability to differentiate between pure and potentially hybridized populations, as well as identify hybrid individuals in the sympatric sites, we combined morphological and genetic data into a bivariate analysis. Those characteristics determined to weigh heavily in the first principle component were combined to produce hybrid index scores. The selected morphological characteristics were normalized so that scores of 0 and 1 represented *B. asteroides*- and *B. decurrens*-like phenotypes, respectively.

Scores were then averaged over traits to produce a morphological hybrid index for each individual. Similarly, the selected genetic characteristics (i.e., the independent probability of assignment to each genealogical class) were combined to produce a genetic hybrid index. The genetic hybrid index for each individual was obtained as the vector distance measuring the cumulative deviation of its independent likelihood for each class from the mean *B. decurrens* values, calculate for the pure GL population. That is, for each sample individual i with independent probability x_{ij} of belonging to genealogical class j, the genetic hybrid index G_i was calculated as $G_i=[\Sigma(x_{ij}-y_j)^2]^{1/2}$, where y_j is the mean independent probability of belonging to genealogical class j in the GL population. This index provides a measure of how closely an individual resembles pure *B. decurrens*.

In order to determine if both morphological and genetic data are required to distinguish between *B. decurrens*, *B. asteroides*, and hybrid individuals, the morphological and genetic hybrid indices were analyzed in a bivariate analysis. For each individual sampled, the morphological hybrid index was plotted against the genetic hybrid index. The distribution of individuals within and among populations was analyzed by constructing 95% confidence ellipses using the bivariate analysis procedure in JMP (version 4.0.4, SAS Institute, 1994). This analysis tests for differences among populations, as well as identifying putative hybrid individuals as outliers from its population of origin.

Results

Morphological analyses

Eight of the ten morphological characteristics measured differed significantly between *B. decurrens* and *B. asteroides* (Table 2). Only leaf length and leaf margin did not differ significantly between species. The presence of leaf decurrence on one sample of *B. asteroides* from the SL population indicates that this characteristic is not completely diagnostic for taxonomic identification.

Genetic analyses

Quantification of genetic variation within populations

Results of the allozyme analysis reveal substantial genetic variation within populations and species (Table 3). One uncommon unique allele (frequency of 0.054) was identified at the SKDH1 locus in *B. asteroides.* Otherwise differences between species were in terms of the frequencies of shared alleles (Appendix 1).

Population-level estimates of contemporary hybridization

Results of the population-level analysis from the putatively hybridizing populations at BT revealed the majority of individuals to be *B. asteroides* (P2), with low frequencies of P1 and BP2 individuals (Table 4). Population-level analysis of the putatively hybridizing populations at F identified the majority of individuals as *B. decurrens* (P1) with a small proportion of individuals assigned to the F2 genealogical class.

Identification of hybrid individuals

Individual assignment tests conducted on the putatively hybridizing populations rejected the null hypothesis of assignment to the *B. decurrens* parental class for three out of a total of 94 individuals. Two of the individuals were from the BT site (samples BT30 and BT34) while the third individual was from the F site (sample F14). For sample BT30, further tests failed to reject the alternative hypothesis of assignment to the *B. asteroides* (P2) class. For samples BT34 and F14, further tests rejected the alternative hypothesis of assignment to the *B. asteroides* (P2) class as well as the F1 hybrid class, but failed to reject assignment to the F2 genealogical class.

Results of the power analysis for the individual assignment tests reveal considerable overlap in the expected distribution of test statistics for all six genealogical classes. Based on these distributions, our power to correctly reject the null hypothesis of membership in the *B.*

decurrens (P1) class is 41.5% of *B. asteroides* individuals, 17.1% of F1 individuals, 19.2% of F2 individuals, 10.1% for BP1 individuals, and 28.7% for BP2 individuals.

Combined morphological and genetic analysis

Principle components analysis

The first two principle components (PCs) explained 54% and the first nine PCs explained 95% of the variance in the combined data set (Table 5). The first principle component (PC1) was most heavily weighted by two morphological characteristics, the decurrence:internode ratio and the number of disc flowers, and four genetic characteristics, the probabilities of assignment to the P1, P2, BP1 and BP2 genealogical classes. The second principle component was heavily weighted by one morphological characteristic (leaf length) and four genetic characteristics, the probabilities of assignment to P2, F1, F2, and BP1 genealogical classes.

Bivariate analysis

The morphological index for each individual was calculated from the normalized decurrence:internode ratio and number of disc flowers. Both the mean morphological indices (SL = 0.16, GL = 0.54, $p<0.001$) and the mean genetic indices (SL = 0.42, GL = 0.21; $p<0.001$) differed significantly between allopatric populations. The mean genetic indices for the allopatric populations . No significant difference was found between the four sites based on the 95% density ellipses around the combined morphological and genetic indices for each individual (Fig. 2). Near-complete overlap of the *B. asteroides* and BT samples, as well as the *B. decurrens* and F samples was observed. Three individual samples were identified as outliers from the site from which they were collected: BT1, F32, and F35.

Discussion

One goal of this study was to develop protocols to differentiate between pure stands of *B. decurrens* or *B. asteroides* and sympatric, putative hybrid populations in order to monitor threats to *B. decurrens*, a federally threatened floodplain species. Our analysis indicates that morphological characteristics provide greater power than allozyme markers to distinguish between *B. decurrens* and *B. asteroides*, although allozyme analysis can differentiate between the species at the population level. Genetic analysis may be more appropriate than morphological characteristics for detecting later generation hybrids or backcross individuals, however. Both morphological and genetic characteristics contributed to the first principle component, which explained 43% of the variation in the correlation matrix. The morphological characters of decurrence:internode ratio and number of disc flowers contributed similar weights to the first PC, and provide inexpensive, near-instant identification between pure species. The four genetic components also contributed similar absolute weights (although opposite signs for each species) to the analysis. The similarity to the weights for the pure parental species and the backcross to either species is likely a consequence of the low statistical power of the genetic analysis to distinguish between individuals of the two genealogical classes.

We find little evidence of current hybridization between these species in the two sympatric populations studied. Two morphological characteristics, the ratio of decurrence:internode length and the number of disc flowers, provide high power (>0.80) to distinguish between *B. decurrens* and *B. asteroides*. The morphological hybrid index score calculated from these measurements provided diagnostic power (100%) to distinguish between these species. Based on this morphological hybrid index, no intermediate morphologies were observed at either sympatric population, indicating that F1 or early generation hybrid individuals are rare or absent. Given the potential for hybrid individuals to lack an intermediate morphology due to either dominance or transgressive hybrid character states (Schwarzback, Donovan, and Rieseberg, 2001), and the near-continuous distribution of the character states we observed, we cannot rule out the presence of hybrid individuals based on these two morphological characters alone.

The genetic analysis based on allozyme data is consistent with the morphological data in failing to identify F1 hybrid individuals. The population-level analysis estimated a zero

frequency of F1 individuals for both sympatric populations (see Table 4). In addition, the individual assignment tests rejected the null hypothesis of being *B. decurrens* in origin for only three individuals, with further tests assigning only one sample to the *B. asteroides* parental class. The remaining two individuals were rejected under both alternate hypotheses of being *B. asteroides* or F1 in origin, but subsequent analyses failed to reject assignment to the F2 genealogical class. Given the power of the individual assignment tests to distinguish between the *B. decurrens* parental class and the F1 hybrid class, it is possible that some proportion of the sampled individuals were F1 in origin but went undetected by our analysis. As described in Nason, Heard and Williams (in press), the number of detected (x) and undetected (N_{F1}-x) F1 individuals is bimodally distributed. When no F1 samples are observed (x=0), the upper 95% confidence limit on the number undetected in the sample is $\max N_{F1}=\ln(\alpha)/\ln(\beta_{F1})$, where α is the experiment-wise error rate and β_{F1} is the power to detect F1 individuals. Given the power to detect F1 hybrids from pure *B. decurrens* in this analysis (0.415) and α=0.05, we calculate the maximum number of undetected F1 individuals sampled at either BT or F to be 3. However, the failure of any individual to be assigned to the F1 genealogical class is consistent with low or zero frequencies of hybridization currently occurring between *B. decurrens* and *B. asteroides* at these sites.

The observed structure of the two sympatric sites is consistent with our *a priori* predictions based on field observations. The BT population more closely resembles *B. asteroides* than *B. decurrens* (see Figure 2), as was expected by the recent increase in *B. asteroides*-like individuals at that site. The number of *B. asteroides*-like genotypes observed at the BT population was lower than may be expected given the morphological observations, as only one individual (sample BT1) expressed a phenotype consistent with *B. decurrens* (see Figure 2). These inconsistencies between genetic and morphological observations may be evidence of the introgression of *B. decurrens* genotypes into the *B. asteroides* population at BT, but more powerful genetic markers are be required to confirm this hypothesis.

The observed structure of the F population was also consistent with *a priori* predictions that it would more closely resemble *B. decurrens*. Although morphological data cannot differentiate between the F population and the pure *B. decurrens* population at GL, the population-level genetic analysis indicates that a small proportion of F2 or later

generation progeny may be present in the F population (see Table 4). In addition, one individual (F14) was identified as being F2 or later generation in origin based on individual assignment tests. This evidence for hybridization events (second generation or later) supports the hypothesis that *B. asteroides* is present in the F population, although this investigation failed to detect a pure *B. asteroides* individual based on either morphological or genetic analysis.

Taken together, these data reveal a potential for interspecific matings between *B. decurrens* and *B. asteroides* under natural conditions. Although the morphological hybrid index failed to identify intermediate phenotypes indicative of hybrid individuals, the genetic analysis based on allozyme markers reveals low levels of later-generation hybrids in both sympatric populations. Population-level analyses of twelve additional *B. decurrens* populations reveal low frequencies of F2 and later generation progeny in four populations (J. DeWoody, Iowa State University, unpublished data). Although no *B. asteroides* have previously been observed at these sites, these data indicate that hybridization between these species may be occurring at a low frequency in other populations as well. Further studies confirming this potential hybridization and investigating the potential for introgression of *B. asteroides* genotypes into *B. decurrens* using more powerful molecular markers are warranted.

The procedures used in this study provide insight for current management and monitoring efforts. The ratio of leaf decurrence: internode length provides diagnostic power to distinguish between pure *B. decurrens* and *B. asteroides* individuals in the field. However, given the range of values observed in *B. decurrens* and that hybrid progeny may display morphologies intermediate or transgressive to the parental species, this single characteristic may not be sufficient to identify hybrid, backcrossed or introgressed individuals. Combining the two morphological characteristics with genetic data describes the majority of the variation between the two parental species, as depicted by the principle components analysis. Although the molecular analysis provides evidence of historic hybridization between *B. decurrens* and *B. asteroides*, no direct evidence of current hybridization (i.e., F1 progeny) was observed. Given the low statistical power of the genetic tests, we cannot rule out the existence of F1 progeny, nor can we confirm the past hybridization events that may be

revealed by our allozyme data. The development of more powerful genetic markers (e.g., microsatellite or sequence data) for these species could greatly increase the precision and power of these analyses for future monitoring efforts. Even with low levels of hybridization between these species, *B. decurrens* maintains genetic and morphological integrity in these populations, and, as observed at Beardstown (BT), where the *B. decurrens* populations appears to be declining due to environmental conditions rather than genetic swamping by *B. asteroides*, the more proximate threat to the populations' survival is insufficient disturbance (flooding) to maintain population regeneration.

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editors. Southwestern Rare and Endangered Plants: Proceedings of the Second Conference. USDA Forest Service, Flagstaff, Arizona, USA.

Table 1. Name, location, and sample sizes for the two allopatric, putatively pure, and two sympatric, putatively hybridizing *Boltonia* study sites. Sample sizes for GL and SL indicate the number of individuals sampled for morphological and for genetic analyses. See text for site descriptions and Fig. 1 for site locations along the Illinois River.

Population	Location	Coordinates	Sample size
Allopatric B. decurrens			
GL	Jersey Co., IL	38° 58' N, 90° 30' W	24, 48
Allopatric B. asteroides			
SL	Calhoun CO., IL	39° 00' N, 90° 33' W	24, 48
Sympatric sites			
BT	Cass Co., IL	40° 01' N, 90° 27' W	47
F	Schuyler Co., IL	40° 04' N, 90° 26' W	47

Table 2. Results of morphological analyses of pure populations of *B. decurrens* and *B. asteroides*. See text for descriptions of the morphological characters measured. Mean values for each species were determined from measurements of 24 individuals sampled from each pure population. Range of observed values are reported in parentheses. For leaf texture, g = glaucous, i = intermediate, t = tough. For leaf margin, e = entire, t = toothed. Power quantified the ability to discriminate between *B. decurrens* and *B. asteroides* based on that single character.

Morphological Character	*B. decurrens* at GL	*B. asteroides* at SL	Power
Decurrence:internode ratio[b]	1.28 (0.54-4.82)	0.0 (0.0-0.06)	1.000
Number of disc flowers[b]	260.0 (185-369)	169.7 (125-204)	0.833
Capitulum height (mm)[b]	1.88 (1.3-2.2)	1.29 (0.8-1.8)	0.667
Capitulum width (mm)[b]	2.58 (1.8-3.6)	2.08 (1.6-2.8)	0.625
Leaf texture (# obs.)[b]	g=24	g=13	
	i=0	i=6	0.458
	t=0	t=5	
Number of ray flowers[b]	48.79 (33-66)	40.54 (28-54)	0.417
Leaf margin (# obs.)	e=6	e=11	0.208
	t=18	t=13	
Avg. disc flower length (mm)[a]	2.86 (2.6-3.2)	2.96 (2.8-3.3)	0.167
Leaf width (mm)[a]	14.9 (6.5-16.5)	1.23 (7.0-16.5)	0.042

[a],[b] means differ significantly at $\alpha = 0.05$, $\alpha \leq 0.0001$, respectively

Table 3. Genetic diversity statistics for *Boltonia decurrens*, *B. asteroides* and two putative hybrid populations, BT and F. N = number of samples, A = mean alleles per locus, A_p = mean alleles per polymorphic locus, P = percent polymorphic loci, H_e = expected equilibrium heterozygosity. *B. decurrens* values were calculated as means of population-level estimates for 13 populations spanning the range of the species.

	N	A	A_p	P	H_e
B. decurrens	874	2.314	2.804	72.34	0.314
B. asteroides	48	2.667	3.000	83.33	0.300
BT	48	2.583	2.900	83.33	0.316
F	48	2.417	3.125	66.67	0.319

Table 4. Genealogical class frequency estimates for two sympatric and potentially hybridizing populations of *B. decurrens* and *B. asteroides*. P1 = *B. decurrens*, P2 = *B. asteroides*, F1 = first-generation hybrid, F2 = second-generation hybrid, BP1 = first-generation backcross to *B. decurrens*, and BP2 = first-generation backcross to *B. asteroides*. See text for details.

	P1	P2	F1	F2	BP1	BP2
BT	0.0700	0.9135	0.0000	0.0000	0.0000	0.0165
F	0.9120	0.0000	0.0000	0.0880	0.0000	0.0000

Table 5. Principle components describing the variation among *B. decurrens* and *B. asteroides*. The eigenvectors (either positive or negative) are listed for each morphological and genetic (probability of being assigned to each genealogical class) characteristic. The percent of variation explained by each PC is listed in parentheses.

Characteristic	PC1 (42.9)	PC2 (11.0)
Decurrence:node ratio	0.312	0.057
Leaf length	0.167	-0.287
Leaf width	0.157	-0.181
Leaf margin	0.089	-0.014
Leaf texture	0.178	0.188
Number ray flowers	0.233	-0.125
Number disc flowers	0.324	-0.093
Captiulum width	0.271	-0.100
Capitulum height	0.281	0.003
Disc flower length	-0.097	-0.048
Prob. *B. decurrens*	0.347	-0.018
Prob. *B. asteroides*	-0.305	-0.381
Prob. F1	-0.199	0.437
Prob. F2	-0.041	0.631
Prob. BP1	0.336	0.267
Prob. BP2	-0.349	-0.060

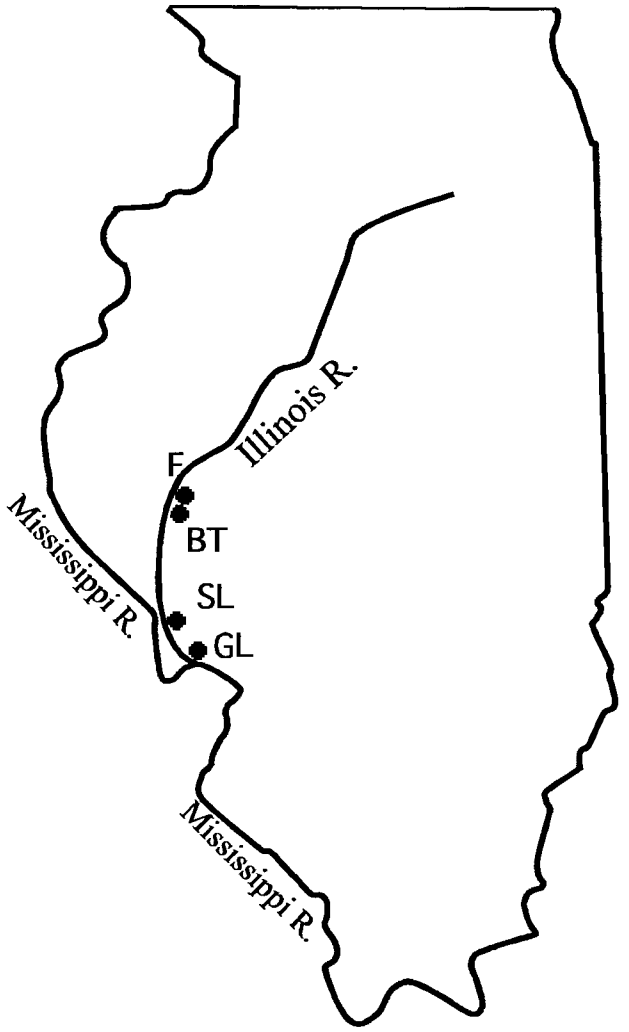

Figure 1. Locations of allopatric and sympatric (hybridized) of *Boltonia decurrens* and *B. asteroides* study sites in Illinois. Pure *B. decurrens* and *B. asteroides* individuals were sampled from Gilbert Lake (GL) and Stump Lake (SL), respectively. The two species occur in sympatry at Beardstown (BT) and Frederick (F), where they form putative hybrid stands. See Table 1 for detailed location information.

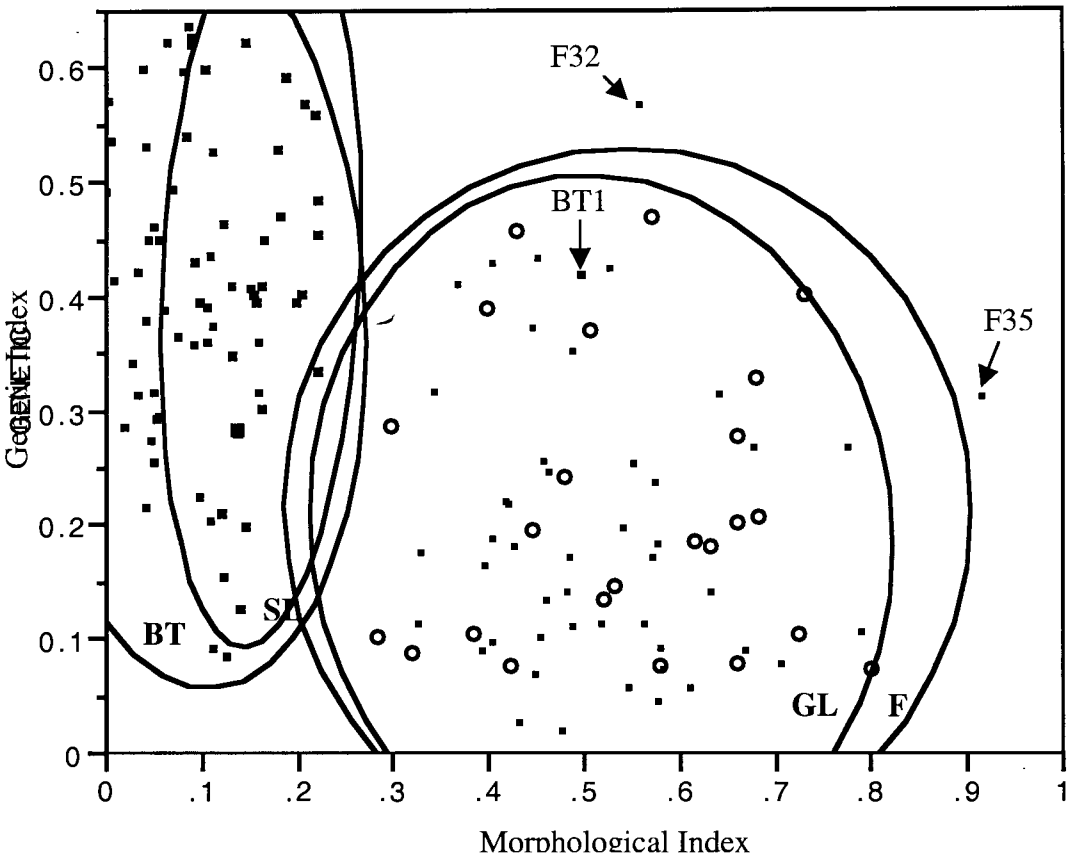

Figure 2. Combined morphological and genetic analysis for two allopatric and two sympatric sites. GL is the allopatric *B. decurrens* population (individuals designated by open circles). SL is the allopatric *B. asteroides* population (X). BT is the sympatric site recently dominated by *B. asteroides* (open squares). F is the sympatric site where *B. asteroides* has only recently been observed (small squares). 95% density ellipses are indicated for each site.

Appendix 1. Allele frequencies for 12 allozyme loci estimated for the parental species *Boltonia decurrens*, *B. asteroides* and two putative hybrid populations. *B. decurrens* estimates represent universal allele frequencies from 14 pooled populations. *B. asteroides* estimates obtained from a single population (SL). The two putative hybrid populations are Beardstown (BT) and Frederick (F).

Locus	*Boltonia decurrens* (universal)	*Boltonia asteroides* (SL)	BT	F
DDH				
2	0.0559	0.1053	0.2368	0.1571
4	0.7364	0.8947	0.7368	0.5714
6	0.2077		0.0263	0.2714
FE				
2	0.1844	0.0349	0.0568	0.1512
4	0.5250	0.5581	0.5341	0.3488
6	0.2863	0.3488	0.3182	0.4884
8	0.0044	0.0581	0.0909	0.0116
IDH				
2	0.7733	0.7093	0.6848	0.8816
4	0.1215	0.1047	0.0870	
6	0.1053	0.1860	0.2283	0.1184
ME				
2	0.3790	0.6354	0.7065	0.1979
3	0.5254	0.3438	0.2391	0.7812
4	0.0955	0.0208	0.0543	0.0208
PGD2				
4	1.0000	1.0000	1.0000	1.0000
PGD3				
2	0.4980	0.4688	0.4149	0.2979
4	0.2392	0.2500	0.2234	0.1702
6	0.2627	0.2812	0.3617	0.5319
GPI				
4	0.9243	0.9062	0.9681	0.7812
6	0.0757	0.0938	0.0319	0.2188
PGM				
2	0.0205	0.0444	0.0109	
4	0.9795	0.9556	0.9891	1.0000

Appendix 1. Continued.

Locus	*Boltonia decurrens* (universal)	*Boltonia asteroides* (SL)	BT	F
SKDH				
1		0.0543		0.0114
2	0.4746	0.7283	0.6556	0.2500
3	0.4188	0.1630	0.2556	0.6932
4	0.0019			
5	0.0907	0.0543	0.0889	0.0455
6	0.0140			
TPI1				
4	1.0000	1.0000	1.0000	1.0000
TPI2				
2	0.3781	0.5532	0.4574	0.4479
4	0.4306	0.3617	0.4255	0.1458
6	0.1619	0.0319	0.0638	0.3021
8	0.0294	0.0532	0.0532	0.1042
UGPP				
2	0.0086	0.0104	0.0213	
4	0.9546	0.9896	0.9787	1.0000
6	0.0368			

CHAPTER 4. GENERAL CONCLUSIONS

Implications for Management and Conservation

An understanding of a variety of processes is required to develop successful protocols to conserve threatened species. Previous studies of *Boltonia decurrens* have revealed that its decline is due to habitat destruction and the suppression of flooding. The goals of this project were to: 1. Investigate the importance of metapopulation dynamics to the genetic structure of this species. 2. Determine if *B. decurrens* hybridizes with *B. asteroides*, a widespread congener, and, if so, describe the structure of the resulting hybrid zone. 3. Create relevant management recommendations based on these conclusions.

The genetic structure of *B. decurrens* is consistent with that expected from frequent local extinction and colonization events, confirming that this species currently persists as a metapopulation. This conclusion has two implications for management protocols. First, propagules (seeds) must disperse regularly in order to maintain sufficient rates of colonization throughout the metapopulation. Previous studies have demonstrated that the achenes produced by *B. decurrens* are adapted for hydrochory (water dispersal). This study emphasizes the importance of flooding to maintain sufficient seed dispersal as a mechanism for colonization. Second, successful management protocols must recognize the importance of unoccupied habitat to the long-term persistence of this species, and must protect unoccupied habitat accordingly.

In addition to confirming the importance of metapopulation dynamics to the structure of this species, this study indicated that a small number of populations most likely contribute to colonization events. If mixing of seed along the river creates a panmictic population, little genetic differentiation would be detected among populations. However, the significant allele frequency variation among populations indicates that colonization processes in *B. decurrens* more closely resembles the propagule pool model than the migrant pool model. In order to maintain similar genetic structure among populations, any attempts to establish new populations of *B. decurrens* should use seed from one or a few populations. The lack of local ecotypes and regional substructure across the population indicate that this seed need not be

collected from neighboring populations. The potential for long-distance seed dispersal in *B. decurrens* may justify the use of seed from distant populations with some frequency.

The second goal of this project was to investigate the potential for interspecific hybridization in two sympatric populations of *B. decurrens* and *B. asteroides*. None of the samples analyzed from either population displayed intermediate morphologies or genotypes indicative of first-generation hybrids. However, detailed genetic analysis revealed low levels of second generation and backcross progeny, indicating that hybridization may be occurring at some low frequency in each population. Ultimately, these conclusions are clouded by the relatively low resolution provided by allozyme variation in these species, and the use of more powerful analyses is advised to confirm these results. Even with low levels of hybridization between these species, *B. decurrens* maintains genetic and morphological integrity in these populations, and, as observed at Beardstown (BT), where the *B. decurrens* populations appears to be declining due to environmental conditions rather than genetic swamping by *B. asteroides*, the more proximate threat to the populations' survival is insufficient disturbance (flooding) to maintain population regeneration.

Finally, *Boltonia decurrens* has proven to be a fascinating organism whose life history is tied to one of the most threatened ecosystems in North America: the great river floodplain. A recurring theme of all studies into the demographics, genetics, and physiology of *B. decurrens* is the importance of regular flooding to the survival of this species. Ultimately, the species' persistence may require a return of the historic flood regimes characteristic of the Illinois River. A better understanding of how these genetic and demographic processes are related to hydrologic events will aid in applying the knowledge gained from studying *B. decurrens* to the protection of organisms with similar life histories.

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