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Inferring demographic processes from the genetic structure of a metapopulation of *Boltonia decurrens* (Asteraceae)

Jennifer DeWoody[1,†,*], John D. Nason[1] & Marian Smith[2]
[1]*Department of Ecology, Evolution, and Organismal Biology, Iowa State University, 353 Bessey Hall, Ames, IA 50011-1020, USA;* [2]*Department of Biological Sciences, Southern Illinois University, Edwardsville, IL 62026-1651, USA;* [†]*Current address: USDA Forest Service, National Forest Genetics Laboratory, 2480 Carson Road, Placerville, CA 95667-5107, USA; (*Author for Correspondence, Fax: +1-530-622-2633; e-mail: jdewoody@fs.fed.us)*

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Abstract

Boltonia decurrens (Asteraceae), a federally listed, threatened floodplain species, requires regular flooding for suitable habitat and seed dispersal. Flood suppression and habitat destruction have resulted in fewer than 25 populations remaining throughout its 400 km range. Because individual populations are widely interspaced (>10 km) and subject to frequent extinction and colonization, seed dispersal along the river, not pollen flow, is likely the primary determinant of population genetic structure. We used neutral genetic markers (isozymes) assayed for fourteen populations to determine which demographic processes contribute to the genetic structure of *B. decurrens*. Significant genetic differentiation was detected among populations ($F_{ST} = 0.098$, $P < 0.05$) but not among regions ($F_{RT} = 0.013$, $P > 0.05$), suggesting that long-distance dispersal events occur and involve seed from a small number of populations. Correspondingly, we found no evidence of isolation by distance, and admixture analyses indicate that colonization events involve seed from 3 to 5 source populations. Individual populations exhibited high levels of fixation (mean $F_{IS} = 0.192$, $P < 0.05$), yet mean population outcrossing rates were high ($t_m = 0.87$–0.95) and spatial autocorrelation analyses revealed no fine-scale within population structure, indicating that inbreeding alone cannot explain the observed fixation. Rather, genetic bottlenecks, detected for 12 of 14 populations, and admixture at population founding may be important sources of fixation. These observations are consistent with a metapopulation model and confirm the importance of regular flooding events, capable of producing suitable habitat and dispersing seed long distances, to the long-term persistence of *B. decurrens*.

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 and Barrett 1996). The ephemeral nature of populations within a metapopulation emphasizes the importance of unoccupied habitat to the survival of the species (Hanski and Simberloff 1997; Hanski 1998), and necessitates a better understanding of landscape-level processes (e.g., dispersal and migration) for the implementation of successful conservation strategies. Ultimately, an understanding of the factors influencing the colonization and extinction of populations, including genetic dynamics (e.g., relatedness of founders of new populations) is required for successful conservation of a metapopulation (Schemske et al. 1994; 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 and Barrett 1996; Giles and Goudet 1997; Thrall et al. 1997).

One demographic process in a metapopulation that can be inferred from its genetic consequences and should be considered when designing conservation plans is the source of colonists in new populations. Colonization occurring by migrants originating from a single population (Slatkin's (1977) propagule pool model), is expected to increase the genetic differentiation among populations (Wade and McCauley 1988). At the other extreme, colonization occurring by migrants randomly chosen from all potential source populations (Slatkin's (1977) migrant pool model) may increase or decrease differentiation among populations, depending on the ratio of colonists to migrants (Wade and McCauley 1988). Although the actual correlation among colonists is expected to fall between these two extremes (Wade and McCauley 1988), describing colonization in the context of these models may provide insight into critical demographic processes which could be used to guide future conservation or restoration efforts. For example, restoration activities should take into account the colonization dynamics of the species, limiting mixing of source populations when a species is best described by the propagule pool model but not when it mimics the migrant pool model.

In addition to metapopulation dynamics, the mechanism of dispersal may influence the genetic structure of a species. For a metapopulation of a plant species that distributes seed via water (hydrochory), dispersal patterns due to hydrochory may better explain the genetic structure among populations than the metapopulation dynamics. Previous studies have shown that hydrochory can result in long-distance seed dispersal events among populations (Kudoh and Whigham 1997; Akimoto et al. 1998). In addition, the tendency for seed dispersal to occur in a downstream direction can also result in the non-random distribution of genetic diversity among populations due to the greater likelihood of new alleles to migrate into downstream rather than into upstream populations (Akimoto et al. 1998; Gornall et al. 1998).

Boltonia decurrens (Torr. and Gray) Wood (Asteraceae), a federally threatened herb, is native to the Illinois River floodplain and an area near the confluence of the Illinois and Mississippi Rivers. As a floodplain specialist and early-successional species, this species relies on regular flooding not only for seed dispersal (Smith and Keevin 1998), but also for the generation of acceptable habitat for new populations (Schwegman and Nyboer 1985; U. S. Fish and Wildlife Service 1990). Without further disturbances, established populations are susceptible to extinction within 5 years of establishment as later-successional species encroach upon the population (Smith et al. 1993). This frequent local extinction and colonization of *B. decurrens* populations creates a metapopulation along the Illinois River (as defined by Hanski and Gilpin 1991, but see Hanski and Simberloff 1997).

Gene flow among plant populations usually occurs via seed or pollen dispersal. Seeds from *B. decurrens* have been shown to have the potential to float long distances, remaining buoyant for up to 4 weeks in laboratory tests (Smith and Keevin 1998). A variety of potential pollinators have been observed on *B. decurrens*, including bees, flies, gnats, and wasps, although the effectiveness of these species as pollinators is not known. Due to the geographic isolation of populations (tens of km), interpopulation movement by the generalist pollinators of *B. decurrens* is likely to be rare. As a result, gene flow via seed dispersal rather than pollen dispersal is expected to best explain the genetic structure of this species.

The decline of *B. decurrens* during the past century due to the channelization of the rivers (Bellrose et al. 1983), and urban and agricultural development across the river floodplain (Schwegman and Nyboer 1985; U. S. Fish and Wildlife Service 1990) has resulted in its listing as a threatened under the U. S. Endangered Species Act (U. S. Fish and Wildlife Service 1988). 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 and Keevin 1998; Smith and Moss 1998), as well as those factors contributing to the local extinction of populations (U. S. Fish and Wildlife Service 1990; Smith et al. 1993). However, a greater understanding of the interpopulation dynamics of this species, such as processes governing the establishment of new populations, would aid management efforts currently underway (Smith 1994).

Given the life history of *B. decurrens*, we predict that both metapopulation dynamics and anisotropic (asymmetric) seed dispersal will significantly influence geographic patterns of genetic variation in the species. We evaluated these hypotheses using a battery of putatively neutral genetic markers (allozymes). Specifically, we address four questions. (1) Is the genetic structure observed among populations consistent with that expected in a metapopulation? (2) Is seed dispersal in *B. decurrens* better described by the migrant pool or the propagule pool model? (3) Does hydrochory result in anisotropic gene flow in this species? (4) How might these findings influence future conservation strategies?

Methods

Study species

B. decurrens is an early-successional species that colonizes newly cleared habitat following flooding events. Seeds require light and wet soil to germinate, and while recruitment of seedlings can be high in suitable new habitat, it is low within established populations (Smith and Keevin 1998). Recent studies of the potential for this species to persist through a seedbank have revealed low germination from soil core samples (0–6%; Redmond 1993) and 0% germination in buried seed (Smith, unpublished data). Seedling rosettes bolt in the spring either the same year as germination or the following season, while basal rosettes, produced in the fall, over-winter and bolt the following summer. Each bolting stem produces an average of ca. 50,000 achenes (Smith and Moss 1998). Successive establishment of basal rosettes around a mother stem eventually leads to the death of the genet due to competition for resources (Smith, pers. observation), resulting in an average lifespan of 5 years per genet. Further, individual plants are shade- intolerant, resulting in the local extinction of populations within 5 years in the absence of further disturbance events. This recurrent colonization and extinction results in a population structure best characterized as a metapopulation.

Due to the ephemeral nature of *B. decurrens* populations, the precise location and size of each population varies from year to year. Currently, 23 populations are known to occur along a 400 km stretch of the Illinois River and the adjoining Mississippi River, of which fourteen populations were sampled for this study (Figure 1 and 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 (Figure 1 and Table 1).

Allozyme analysis

Samples were collected in the field in May, 2000, and May and September, 2001. Approximately 0.06 g of leaf tissue was collected from each individual, frozen in liquid nitrogen, and stored at −80 °C until processed. Total protein was extracted by grinding samples in 0.5 ml of crushing buffer (Phosphate Buffer of Soltis et al. (1983) modified to contain 6% w/v PVP) and absorbing the solution onto 3 mm × 8 mm Whatman®paper wicks. Wicks were stored at −80 °C until electrophoresis.

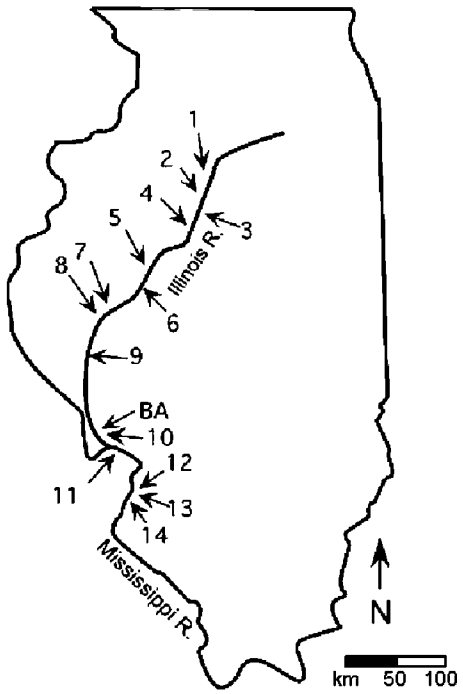

Figure 1. Locations of the 14 populations of *Boltonia decurrens* sampled for this study. These study sites include the majority of populations currently known to exist and encompass the natural range of the species. BA indicates the single population of *Boltonia asteroides* sampled as an outgroup.

Table 1. Details on the 14 *B. decurrens* and one *B. asteroides* populations sampled in this study

No	Name	Location	Latitude, Longitude	N	Year
B. decurrens					
1[b]	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[a]	Woodford County Conservation Area	Woodford Co., IL	40°53′ N, 89°27′ W	18	1994
4	McClugage Bridge	Peoria Co., IL	40°43′ N, 89°33′ W	96	1989
5[c]	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[b]	Meredosia Lake	Morgan Co., IL	39°52′ N, 90°33′ W	48	1984
10[b]	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[a]	Horseshoe Lake	Madison Co., IL	38°41′ N, 90°06′ W	96	1994
13[a,c]	Waste Management	Madison Co., IL	38°40′ N, 90°07′ W	95	1994
14	Fairmont City	St. Clair Co., IL	38°39′ N, 90°07′ W	48	1984
B. asteriodes					
BA	Stump Lake	Jersey Co., IL	39°00′ N, 90°33′ W	48	n.a.

[a] Denotes populations sampled for assignment analysis.
[b] Denotes populations included in the mating system analysis.
[c] Denotes populations sampled for spatial autocorrelation analysis.
Population numbers represent the location of the *B. decurrens* populations along the Illinois River, from upstream to downstream. N indicates sample size used to estimate allele frequencies for each population. Year is the year that the population was first reported, with the first formal survey occurring in 1984.

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 (S8, modified from Soltis et al. (1983) System 8). Samples were assayed for 11 protein stains, resulting in 13 putative loci. Seven loci were resolved in MC6.1: glucose-6-phosphate isomerase (GPI1, EC 5.3.1.9), malic enzyme (ME1, EC 1.1.1.40), 6-phosphogluconate dehydrogenase (PGD2 and PGD3, EC 1.1.1.44), shikimic dehydrogenase (SKD1, 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). Three loci were resolved in S8: dihydrolipoamide dehydrogenase (DDH1, EC 1.8.1.4), fluorescent esterase (FE1, EC 3.1.1.-), and menadione reductase (MNR1, EC 1.6.99.2). All stain recipes were adapted from Wendel and Weeden (1989) except UGPP (Manchenko 1994). All loci produced banding patterns consistent with published protein structures and diploid, Mendelian inheritance.

Data analysis

In order to determine which processes significantly influence the genetic structure of *B. decurrens*, the following analyses were performed with respect to the questions posed above.

Is the genetic structure observed among populations consistent with that expected in a metapopulation?

Quantification of within population genetic diversity
To quantify the genetic structure of *B. decurrens* across the range of the species, samples were collected from fourteen populations (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 (A_P), expected heterozygosity (H_e). In addition, we assessed the presence of unique alleles and high levels of variation (relative to the species mean) to identify

genetically distinct populations that may be of particular concern for conservation. We also calculated the fixation index (F) for each population (a measure of excess homozygosity relative to Hardy–Weinberg proportions) using the program GDA (Lewis and Zaykin 2001).

Tests for recent population bottlenecks As new populations are founded, older populations tend toward local extinction, or populations undergo reductions in size, small effective sizes may result in a genetic bottleneck. We tested for recent genetic bottlenecks in each sample population using the methods of Cornuet and Luikart (1996). In this test, recent genetic bottlenecks are detected as a ''heterozygosity excess'' relative to the heterozygosity expected at mutation-drift equilibrium, given the number of alleles observed at a locus. Populations undergoing recent bottlenecks are expected to lose rare alleles at a greater rate than the loss of heterozygosity. This heterozygosity excess differs from an excess of heterozygotes in that it is based on mutation-drift equilibrium and not Hardy–Weinberg equilibrium, the latter being more quickly recovered via random mating. Tests were implemented using the one-tailed Wilcoxon's test for excess heterozygosity under the infinite alleles model. All tests were implemented using the program Bottleneck (Piry et al. 1999; available at: http://www.ensam.inra.fr/URLB/bottleneck/bottleneck.html).

Demographic sources of fixation A previous study observed significant fixation indices in populations of *B. decurrens* (T. Ranker, unpublished data) while hand-pollination experiments indicate this species is highly outcrossing (< 5% viable seed due to self-pollination, Tofari 2000). Significant fixation indices may be caused by recent mixing of colonists from source populations (a Wahlund effect), or by inbreeding or self- fertilization. We conducted a mating system analysis and a spatial autocorrelation analysis to determine if self-fertilization or biparental inbreeding contribute to observed levels of fixation.

A mating system analysis was conducted to estimate the outcrossing rate (one minus the selfing rate) of *B. decurrens* using progeny arrays for three randomly chosen populations: nos. 1, 9, and 10. For each population, ten seedlings from each of ten mothers were grown from seed collected in 1995. Fresh tissue was assayed for allozyme variation as described above for a subset of polymorphic loci: DDH1, FE1, GPI1, ME1, MNR1, PGD3, SKDH1, and TPI2. Ritland's (2002) multilocus approach was used to estimate the average outcrossing rate per population (t_m).

We tested for significant fine-scale population substructure as a source of significant fixation indices in *B. decurrens* by collecting 60 samples at 1m intervals in two populations found to be geographically large enough to accommodate a 60 m transect. One transect was sampled in population 13 in May 2001; two transects were sampled in population 5, one in May 2000 and one in May 2001. The multilocus genotype for each individual was determined using the complete set of allozyme loci described above. Spatial autocorrelation analyses employing an estimator of kinship (f_{ij}) were used to test for fine-scale population structure at 1 m intervals in each transect according to the methods of Kalisz et al. (2001).

Is seed dispersal in B. decurrens better described by the migrant pool or the propagule pool model? 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 inferred the process of population colonization in *B. decurrens* by estimating the source populations for three of the most recently founded populations in our study sample: nos. 3, 12, and 13, each reported in 1994. These population assignment tests were completed in order to determine if population colonization is more similar to the migrant pool model or the propagule pool model. Collections from each test population were made in May 2001: 96 samples were randomly collected from population 12, 95 from population 13, and all 18 individuals present at population 3. The multilocus genotype of each sample was determined for all allozyme loci assayed using the methods described above.

Given the possibility of random mating subsequent to population establishment for the three *B. decurrens* populations sampled, admixed individuals, potentially containing genes from multiple source populations, may be present in each sample. In order to account for this possibility, and to identify the most appropriate algorithm for this data set, a series of power tests were performed on simulated data created from observed allele

frequencies. For each putative source population (populations 1–2, 4–11, 14), 10,000 individuals were simulated based on the allele frequencies observed for the full set of allozyme data described above. The power of this data set was estimated by completing assignment tests for each of the eleven simulated populations, calculating power as the percentage of individuals assigned to the correct source population. This power analysis was tested for three assignment methods employed by the software GeneClass (Cornuet et al. 1999): Bayesian likelihood, Nei's standard genetic distance, and Cavalli-Sforza distance. All assignment tests were completed using the direct assignment option, which assigned each individual to exactly one source population (Cornuet et al. 1999). The method that correctly assigned the highest percentage of individuals to their source population, averaged over simulated populations, was used to analyze the three test populations (populations 3, 12, and 13).

Following the power analysis, the direct assignment procedure, employing the Bayesian likelihood method, was used to assign individuals from each of the three test populations to exactly one source populations. The set of putative source populations (S) was defined as those populations known to be extant at the time the populations were first reported (Table 1): $S = \{1–2, 4–11, 14\}$.

The distribution of individuals assigned to each source population was tested against the null hypothesis that the process of colonization approximated the migrant pool model for each test population. That is, the number of individuals assigned to each source population was compared to the distribution expected if migrants were chosen at random from each source population. For this data set, a source population is expected to contribute 1/11th, or a proportion of 0.09 of the samples. For each test population, a χ^2 test was used to determine if the number of individuals assigned to each source population was significantly different from that expected under the null hypothesis.

Does hydrochory result in anisotropic gene flow in this species?

Tests of hierarchical genetic structure among populations We used Wright's F-statistics to test two *a priori* models of hierarchical population structure in *B. decurrens* based on the distribution of populations along the Illinois and Mississippi Rivers. The first model assumed no regional structure among populations, resulting in a 2-level hierarchy for which we estimated the fixation index within populations (F_{IS}) and allele frequency variation among populations relative to the total metapopulation (F_{ST}). The second model assumed a 3-level hierarchy, with populations clustered into a northern region located on the upper Illinois River (populations 1 to 9), and a southern region located on the lower Illinois and adjoining Mississippi Rivers (populations 10 to 14). For this 3-level model we estimated the fixation index within populations (F_{IS}), allele frequency variation among populations 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 and Cockerham (1984) as implemented by the program GDA (Lewis and Zaykin 2001). The significance of these estimates was determined by bootstrapping over loci (1000 replicates).

Tests for isolation by distance In order to determine if hydrochory in *B. decurrens* results in long-distance gene flow events, we tested for isolation by distance using the methods of Slatkin (1993) and Rousset (1997). The results of the two tests did not differ, so we present only the Slatkin (1993) analysis here. We calculated $\hat{M}$, Slatkin's (1993) estimate of Nm, the effective number of migrants per generation, from pairwise F_{ST} values estimated using the method of Nei and Chesser (1983) for all possible pairs of populations. We tested for isolation by distance using 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). As per Slatkin (1993), $\hat{M}$ and geographic distance were log-transformed before $\hat{M}$ was regressed onto distance for all possible pairs of populations. The significance of each regression was tested with randomization tests implemented using the program Permute! (http://www.fas.umontreal.ca/BIOL/Casgrain/en/labo/permute/index.html).

Tests for dispersal-based clines in genetic variation We tested the hypothesis that anisotropic gene flow due to hydrochory results in downstream populations containing higher levels of genetic variation than upstream populations by regressing the distance of each population from the most downstream site against standard measures of genetic diversity. The analyses were conducted using both the direct and river distance between populations (estimated as described above), and four measures of genetic diversity: average number of alleles per polymorphic locus (A_p), percent polymorphic loci (P), and expected (H_e) and observed (H_o) heterozygosity for each population. The significance of each regression model was tested via the F-test using JMP (version 4.0.4, SAS Institute, 2000).

Evolutionary relationships among populations We also tested for the genetic consequences of hydrochory by estimating evolutionary relationships among populations. Seed dispersion strongly skewed in a downstream direction is expected to result in a hierarchically nested population phenogram, in which upstream populations are ancestral to downstream populations (Figure 2A). We constructed a population phenogram for 14 *B. decurrens* populations and one *B. asteroides* population 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 (Figure 2A) using the program GeneTree as described by Page (1998).

Results

Is the genetic structure observed among populations consistent with that expected in a metapopulation?

Quantification of within population genetic diversity Eleven of thirteen allozyme loci (84.6%) surveyed were variable and reveal high levels of genetic diversity maintained within individual populations of *B. decurrens* (Table 2). No population contained unique alleles, although several alleles were rare,

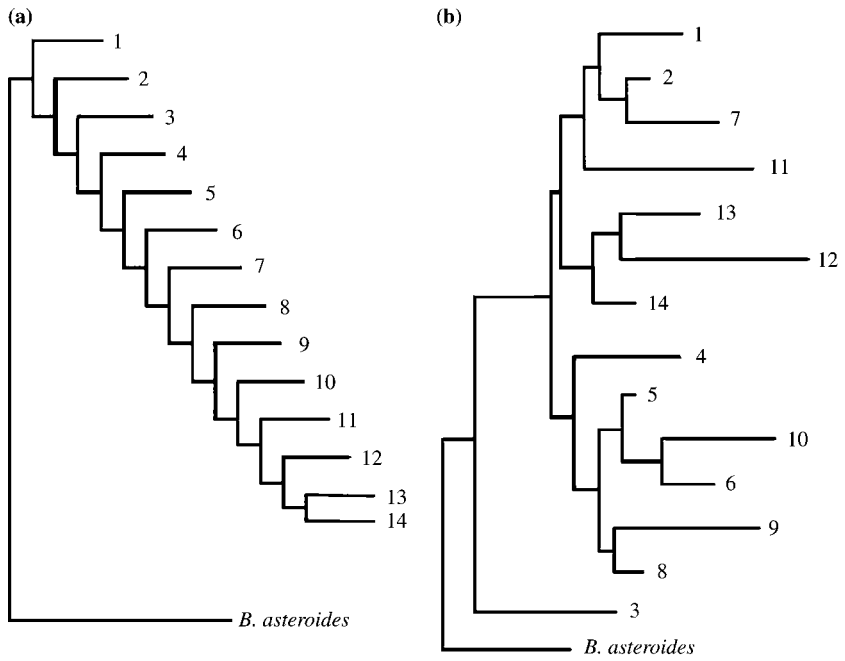

Figure 2. Hypothetical and observed population phenograms for 14 populations of *B. decurrens*. (*a*) Hypothetical phenogram expected as a consequence of unidirectional downstream gene flow due to hydrochory. (*b*) Population phenogram constructed using allozyme data, Nei's genetic distance, and neighbor-joining. Bootstrap support was less than 50% for all inferred relationships among *B. decurrens* populations.

including FE1-8 and PGM1–2, each observed in only three populations (Appendix A). Despite high levels of diversity, seven populations (nos. 1, 4, 5, 8, 10, 12, and 13) exhibited an excess of homozygotes, as indicated by significant fixation indices (mean $F = 0.162$, Table 2).

Tests for recent population bottlenecks Evidence of recent bottlenecks was detected in 12 of the 14 populations tested (86%). The excess of heterozygosity was marginally significant ($P < 0.05$) in five populations (nos. 2, 6, 7, 11, and 14) and highly significant ($P < 0.01$) in seven populations (nos. 1, 3, 4, 5, 9, 10, and 13).

Demographic sources of fixation The results of the mating system analysis indicate that *B. decurrens* is characterized by high levels of outcrossing. Multilocus outcrossing rates ranged from 0.873 (± 0.059) in population 10 to 0.944 (± 0.038) in population 1 and 0.952 (± 0.036) in population 9.

Spatial autocorrelation analyses failed to reveal significant fine-scale population structure within *B. decurrens* populations 5 and 13. Figure 3 shows the results of the spatial autocorrelation analysis of population 13. Results for population 5 were similar.

Table 2. Summary genetic statistics over 13 allozyme loci evaluated for 14 populations of *B. decurrens* distributed across the range of the species

Population	A	A_p	P	H_e	F
1	2.308	2.546	84.62	0.351	0.154*
2	2.385	2.800	76.92	0.309	0.105
3	2.077	2.750	61.94	0.333	0.116
4	2.231	2.600	76.92	0.312	0.081*
5	2.385	3.000	69.23	0.332	0.101*
6	2.167	2.750	66.67	0.276	0.044
7	2.154	2.875	61.45	0.258	0.082
8	2.385	2.800	76.92	0.297	0.208*
9	2.385	2.800	76.92	0.371	0.074
10	2.308	2.889	69.23	0.352	0.157*
11	2.308	2.700	76.92	0.295	0.115
12	2.385	2.800	76.92	0.211	0.142*
13	2.462	2.900	76.92	0.330	0.175*
14	2.462	2.900	76.92	0.322	0.086
Mean	2.314	2.804	72.34	0.314	0.193*
St. Dev.	0.117	0.122	6.675	0.042	0.045

A = mean alleles per locus, A_p = mean alleles per polymorphic locus, P = percent polymorphic loci, H_e = expected equilibrium heterozygosity, and F = population fixation index with * denoting values significantly different from zero.

Is seed dispersal in B. decurrens better described by the migrant pool or the propagule pool model?

Power analyses revealed the Bayesian likelihood method to most accurately assign simulated individuals to the correct source population (unpublished data). For the eleven simulated source populations, power analyses conducted using the Bayesian method provided moderate power to identify most source populations (mean 0.6059, standard deviation 0.1493), with two populations below 50% (nos. 2 = 0.4616 and 5 = 0.2718).

Multiple populations were indicated as sources of propagules for each test population (Table 3). Notably, two different source populations, nos. 6 and 14, contributed the greatest proportion of seed to test populations 12 and 13, respectively, even though these test populations are located in neighboring sites (approx. 4 km apart) and were first recorded the same year. Chi-square tests of the null hypothesis that population colonization was similar to the migrant pool model were highly significant for test populations 12 and 13 ($\chi^2 = 79.516$ and 145.896, respectively; $P < 0.01$) and marginally significant for population 3 ($\chi^2 = 17.406$; $P < 0.05$).

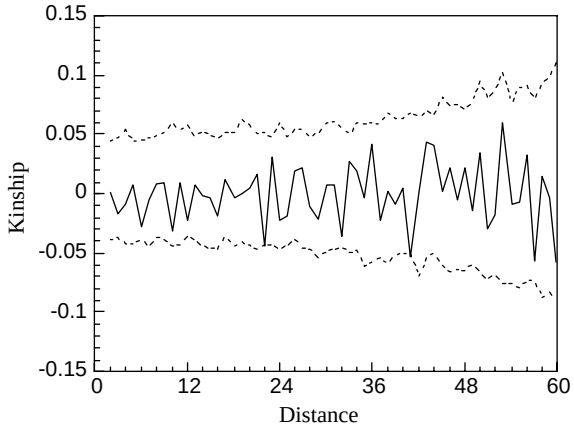

Figure 3. Observed fine-scale population structure in population no. 13. The solid line indicates the estimated kinship (f_{ij}) associated with all possible pairs of individuals collected along a transect. Dashed lines indicate the bootstrap 95% confidence interval around the null hypothesis of no correlation between kinship and distance.

Table 3. Counts of individuals in three recently founded test populations assigned to 14 potential source populations

Source population	Test populations		
	3*	12**	13**
1	2	1	6
2	2	16	1
4	2	3	5
5	1	14	2
6	0	26	3
7	2	10	10
8	0	16	14
9	6	2	3
10	0	0	1
11	1	3	10
14	2	5	40

Distribution of samples among source populations differs significantly from that expected under the null hypothesis of the migrant pool model.
*$P < 0.05$.
**$P < 0.01$.

Does hydrochory result in anisotropic gene flow in this species?

Tests of hierarchical genetic structure among populations Significant differentiation among populations of *B. decurrens* was observed in both the 2-level and 3-level hierarchical models. Differentiation among regions was not significant for the 3-level model ($F_{RT} = 0.013$; $P > 0.05$), however, and variation among populations within regions ($F_{SR} = 0.104$; $P < 0.05$) was consistent with the variation observed among all populations in the 2-level model ($F_{ST} = 0.098$; $P < 0.05$). Within population fixation indices were equivalent for both models ($F_{IS} = 0.192$; $P < 0.05$).

Tests for isolation by distance Regressions of the log effective migrants per generation and both log direct distance ($r^2 = 0.0025$, $P > 0.05$) and log river km distance ($r^2 = 0.0012$, $P > 0.05$) between populations were not significant. No evidence for isolation by distance was found among the fourteen *B. decurrens* populations. Values of $\hat{M}$ were non-zero, however (mean $\hat{M} = 4.55$, std. dev. = 1.99, range 1.51–11.32).

Tests for dispersal-based clines in genetic variation No evidence for non-random distribution of genetic variation indicative of anisotropic gene flow was observed in *B. decurrens*. All analyses produced non-significant ($P > 0.05$) regression coefficients, regardless of the measure of genetic diversity or model of distance from the southernmost population tested.

Evolutionary relationships among populations Phenetic analysis produced a hypothesis of the evolutionary relationships among populations that was poorly supported by the genetic data (Figure 2b). Bootstrap values were less than 50% for all nodes. Tests to reconcile the observed phenogram with the hypothetical phenogram (Figure 2a) showed the observed relationship among *B. decurrens* populations to be no different from random ($P > 0.05$).

Discussion

Is the genetic structure observed among populations consistent with that expected in a metapopulation?

The genetic structure of a metapopulation does not result from a single process, but rather from a number of demographic processes, e.g., local extinction and colonization. Theoretical models predict that recurrent local extinction in particular should lead to a decrease in overall genetic diversity relative to the case where extinction is absent (McCauley 1991). In addition, the source of colonists in newly formed populations is expected to affect levels of genetic differentiation among populations (Wade and McCauley 1988). Based on these predictions, genetic data can reveal which demographic processes influence the genetic structure of a species.

Results of the tests for heterozygosity excesses relative to allelic diversity provide evidence of recent bottlenecks in *B. decurrens* (12 of 14 populations, 86%). Although it is unclear whether bottlenecks occur upon population establishment, during population senescence, or both, it is likely that significant decreases in effective population size in *B. decurrens* are a consequence of this species' reliance on intermittent floods for population establishment and persistence (Smith et al. 1998). These results are also consistent with annual surveys of these populations, which record a net decrease in individuals since the record floods of 1993. These tests provide evidence of bottlenecks

despite the fact that this species may violate two of the assumptions of the Bottleneck algorithm (Cornuet and Luikart 1996): that there is no immigration and no substructure in the sampled populations. However, violations of these assumptions will result in a more conservative test, as both immigration and genetic substructure are expected to increase the number of rare alleles in a population without increasing the heterozygosity, making it less likely to detect a heterozygosity excess relative to the number of alleles. Thus, the results presented here are likely valid estimates of recent genetic bottlenecks in 12 populations of *B. decurrens*.

Although the tests for recent bottlenecks indicate that most populations have undergone decreases in effective population size, the levels of genetic variation observed in *B. decurrens* are relatively high (Table 2), and seem greater than would be expected if frequent local extinctions alone contributed to the genetic structure of the metapopulation. Measures of allelic diversity are expected to reflect historic levels of variation in a species, and these values indicate that large effective population sizes were likely common in *B. decurrens* prior to the species' decline over the past century. This result is consistent with early surveys of the Illinois River, which described *B. decurrens* as a common along a 400 km stretch of the river and its tributaries, and would indicate that reductions in effective population sizes, while present in most populations, are novel, not historical, events for this species. If so, then levels of genetic variation should decline as newly isolated populations continue to undergo recurrent bottlenecks.

In *B. decurrens*, population establishment occurs with propagules drawn from a small number of source populations, and can be described as coordinated colonization (see question 2 below). The pooling of multiple subpopulations into a single sample can result in an apparent excess of homozygotes within a populations (i.e., a Wahlund effect, Hartl and Clark 1997). Under this model, F_{IS} for individual populations should be comparable to F_{ST} for the total population as a result of the Wahlund effect. Although the observed differentiation among populations of *B. decurrens* is less than the mean fixation index ($F_{ST} = 0.098$; $F_{IS} = 0.162$), two of the most recently established populations display a significant fixation index (Populations 12 and 13, Table 2). A Wahlund effect is expected to be broken after a single generation of random mating, with any fixation occurring from admixture resolving to Hardy–Weinberg equilibrium, a situation that may have occurred in these populations of *B. decurrens*. Given both the observation that differentiation among populations is less than the mean fixation index, and that a Wahlund effect is transient, the process of colonization may account for some, but not all, of the fixation observed within individual populations.

In addition to metapopulation processes, demographic processes contribute to the observed levels of fixation. 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. Given that *B. decurrens* is apparently capable of self-fertilization, we ask if this process is sufficient to explain the large fixation indices observed within populations (mean $F_{IS} = 0.162$, range 0.044–0.208). 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* alone could account for fixation indices as great as $F = 0.07$. As this is well less than $F_{IS} = 0.162$ we conclude that the mating system alone cannot explain the observed levels of fixation in *B. decurrens*. As a confirming example, for highly outcrossing populations, fixation is expected to be low, yet in population 1, $t_m = 0.944$ while $F_{IS} = 0.154$ and is significantly greater than zero.

Additional observations support the conclusion that strictly intrapopulation processes are insufficient to explain observed levels of fixation. First, given high rates of population turnover in *B. decurrens*, the component of fixation attributable to the mating system is unlikely to reach its equilibrium value. Second, given the lack of detectable fine-scale genetic structure within populations, 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 summary, the significant fixation indices observed in *B. decurrens* can be explained in part by processes occurring at both the

interpopulation (Wahlund effect) and the intrapopulation (mating system and genetic bottlenecks) levels, although additional as yet unidentified factors likely contribute.

Is seed dispersal in B. decurrens better described by the migrant pool or the propagule pool model?

The process of population establishment in *B. decurrens* resembles the propagule pool model, but is better described as correlated colonization, and likely contributes significantly to the evolution of its genetic structure. Rather than universal mixing of seed during colonization events, newly established populations are founded by propagules originating in a small number of source populations (Table 3). Results of the χ^2 tests support this conclusion, indicating highly significant deviations from the migrant pool model for two test populations (nos. 12 and 13, $P < 0.01$), and moderately significant deviations in population 3 ($P < 0.05$). The significant differentiation among populations (F_{ST}) is also consistent with a process similar to the propagule pool. It is likely, however, that random mating subsequent to population establishment has produced admixed individuals whose phenotypes better match a third, non-source population than either of the two parental source populations. Such an event would be expected to assign individuals to non-source populations in a near-random pattern due to the independent assortment of isozyme loci. Given the large proportion of samples assigned to source populations for test populations 12 and 13 (where sample sizes were large), the evidence is inconsistent with the migrant pool model, even if admixed individuals were misassigned. Based on these observations, the interpopulation genetic structure in *B. decurrens* is likely a consequence of colonization dynamics more similar to the propagule pool model than the migrant pool model of colonization.

Does hydrochory result in anisotropic gene flow in this species?

Hydrochory has been shown to result in long-distance seed dispersal among plant populations (Kudoh and Whigham 1997; Akimoto et al. 1998). 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. Tests for isolation by distance found no evidence that gene flow is restricted geographically in *B. decurrens*. Although the recent genetic bottlenecks mean that it is unlikely this species is in mutation-drift equilibrium (an assumption of Slatkin's (1993) test), additional evidence supports the conclusion that long-distance seed dispersal occurs with some frequency. First, Slatkin (1993) showed that for non-equilibrium populations, the lack of correlation between geographic and genetic distance may indicate a lack of gene flow when values of $\hat{M}$ (a proxy for N_m, the effective number of migrants per generation) are low. In this study, values of $\hat{M}$ were greater than zero (mean 4.55, std. dev. 1.99), indicating that migration occurs regularly. At the other extreme, Slatkin (1993) argued that a lack of isolation by distance coupled with larger values of $\hat{M}$ indicates that the populations were recently founded. This conclusion would be consistent with metapopulation processes occurring in this species. In addition, hierarchical tests of genetic differentiation found no evidence of regional structure in this metapopulation ($F_{RT} = 0.013$; $P > 0.05$). Together, these data indicate that migration occurs at some frequency in *B. decurrens*, either during population establishment or subsequently, and is not restricted geographically.

Previous studies of species predominately distributed by hydrochory also found limited evidence for unidirectional gene flow, as indicated by higher levels of genetic variation in downstream populations (Waser et al. 1982; Akimoto et al. 1998; Gornall et al. 1998). However, we found no significant evidence of downstream populations containing higher levels of genetic variation than upstream populations, indicating that highly anisotropic gene flow does not significantly influence the distribution of genetic variation in *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. Severe flooding downstream can cause backwaters of 160 km 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 et al. 1998), it is possible

that these backflows allow propagules to move in an upstream manner. Populations collected for this study were likely affected by these slow flows or backwaters as recently as 1993 and, to a lesser extent, 1995. Second, the potential for wildlife to act as vectors in 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 and 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, this alternate mode of seed dispersal may be sufficient to maintain equivalent levels of genetic variation across the range of the species.

The population phenogram provides additional evidence that seed dispersal does not only occur in a downstream manner. The topology of the phenogram is not consistent with that expected from a strictly downstream movement of seed, regardless of the lack of bootstrap support (see Nason et al. 2002). The lack of bootstrap support for the tree, indicating a lack of distinct evolutionary relationships among populations, provides additional evidence that these populations were not historically distinct and isolated, which is consistent with the results of the bottleneck tests and observed levels of genetic variation. The placement of population 3 basal to the other *B. decurrens* populations is likely an artifact of the small population size at that site.

How might these findings influence future conservation strategies?

The conclusions drawn from this study should aid in efforts to conserve *B. 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 and 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 to its persistence. 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 evidence of local ecotypes characteristic of different regions within the species' range, indicating that *B. decurrens* is currently functioning as a single evolutionary significant unit and that management protocols should be framed accordingly. Third, the absence of private alleles or local ecotypes and the lack of isolation by distance and regional geographic structure indicate that efforts to reestablish populations of *B. decurrens* could proceed using seed collected from a small number of populations and that such collections need not be restricted to neighboring or upstream sites. Fourth, while we cannot be certain of the degree to which historical levels of genetic variation in *B. decurrens* have already been reduced by human-mediated habitat fragmentation, our data can be used as a baseline against which future levels and geographic patterns of genetic structure can be compared. Finally, the lack of isolation by distance among populations emphasizes the importance of stochastic flooding events sufficient to distribute seed across the range of this species. This conclusion indicates that restoration of historic flooding patterns to the Illinois River floodplain is critical to maintaining the natural processes that have contributed to the evolution of genetic structure in *B. decurrens* and provides the highest likelihood of its long-term persistence.

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Appendix A. Allele frequencies observed at 13 isozyme loci in 14 populations of *B. decurrens*

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DDH1														
2	0.0260	0.0625	0.0882	0.0805	0.1042		0.0625	0.0952	0.0938	0.0227	0.0426	0.0337	0.0056	0.1042
4	0.7917	0.6458	0.5294	0.7356	0.6875		0.8750	0.9688	0.7396	0.6023	0.5638	0.8652	0.7247	0.6979
6	0.1823	0.2917	0.3824	0.1839	0.2083		0.0625	0.0833	0.1667	0.3750	0.3936	0.1011	0.2697	0.1979
FE1														
2	0.3462	0.1556	0.4444	0.0659	0.1458	0.1375	0.4130	0.1889	0.3854	0.0682	0.0625	0.0684	0.1702	0.1702
4	0.4121	0.4667	0.3056	0.3187	0.4062	0.5250	0.4239	0.5333	0.3438	0.4318	0.8333	0.8474	0.6968	0.4574
6	0.2418	0.3778	0.2500	0.6154	0.4062	0.3375	0.1630	0.2778	0.2708	0.4773	0.1042	0.0789	0.1330	0.3723
8					0.0417					0.0227		0.0053		
GPI1														
4	0.8177	0.9167	1.0000	0.9531	0.8750	0.8659	1.0000	0.9688	0.9479	0.6354	1.0000	0.9948	0.9789	0.9792
6	0.1823	0.0833		0.0469	0.1250	0.1341		0.0312	0.0521	0.3646		0.0052	0.0211	0.0208
IDH1														
2	0.6989	0.8409	0.5500	0.8389	0.3958	0.8333	0.8723	0.8587	0.6667	0.4889	0.8958	0.8407	0.6860	0.8286
4	0.1818	0.1250	0.1000	0.1278	0.1458	0.0278	0.0106	0.0109	0.2444	0.1111	0.0729	0.1429	0.1279	0.1143
6	0.1193	0.0341	0.3500	0.0333	0.0521	0.1389	0.1170	0.1304	0.0889	0.4000	0.0312	0.0165	0.1860	0.0571
ME1														
2	0.3229	0.4271	0.5000	0.3830	0.3958	0.2679	0.3125	0.1771	0.1979	0.2717	0.2812	0.6947	0.3617	0.5000
3	0.5469	0.3958	0.3056	0.6011	0.4896	0.5893	0.6042	0.7292	0.4062	0.7283	0.7188	0.2263	0.5745	0.4894
4	0.1302	0.1771	0.1944	0.0160	0.1146	0.1429	0.0833	0.0938	0.3958			0.0789	0.0638	0.0106
MNR1														
2	0.8698	0.9167	0.4706	0.6316	0.7708	0.9600	0.9062	0.6087	0.5729	0.7955	0.6739	0.9521	0.6611	0.7812
6	0.1302	0.0833	0.5294	0.3684	0.2292	0.0400	0.0938	0.3913	0.4271	0.2045	0.3261	0.0479	0.3389	0.2188
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	0.3333	0.3906	0.5521	0.4762	0.5213	0.6170	0.4787	0.4149	0.1296	0.8105	0.6941	0.5233
4	0.5000	0.1979	0.1944	0.1823	0.1875	0.2143	0.4468	0.0851	0.0957	0.2234	0.4444	0.0789	0.2118	0.1047
6	0.3073	0.2812	0.4722	0.4271	0.2604	0.3095	0.0319	0.2979	0.4255	0.3617	0.4259	0.1105	0.0941	0.3721
PGM1														
2	0.0054										0.2128		0.0549	
4	0.9946	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.7872	1.0000	0.9451	1.0000
SKD1														
2	0.6198	0.4674	0.6765	0.6146	0.2766	0.4000	0.4787	0.4062	0.3085	0.3830	0.5729	0.4894	0.4205	0.3511
3	0.3802	0.4674	0.3235	0.3854	0.6809	0.6000	0.2979	0.5104	0.6383	0.5957	0.3438	0.5000	0.1364	0.2872
4										0.0213	0.0104			

Appendix A. (*Continued*).

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5		0.0543			0.0426		0.2234	0.0625			0.0729	0.0106	0.3920	0.3085
6		0.0109						0.0208	0.0532				0.0511	0.0532
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	0.1389	0.3906	0.3690	0.7125	0.1042	0.5109	0.4792	0.4889	0.3372	0.6413	0.3316	0.2396
4	0.4348	0.7128	0.7778	0.1823	0.4167	0.1625	0.7396	0.3696	0.4271	0.4222	0.6395	0.3261	0.3947	0.5938
6	0.3043	0.1915	0.0833	0.4271	0.2024	0.1125	0.1562	0.1196	0.0104	0.0444	0.0233	0.0326	0.1474	0.0729
8					0.0119	0.0125			0.0833	0.0444			0.1263	0.0938
UGPP1														
2								0.0312				0.0417		0.0312
4	0.8526	0.9583	1.0000	0.9844	1.0000	1.0000	1.0000	0.9688	0.7500	1.0000	0.9896	0.9583	1.0000	0.9688
6	0.1474	0.0417		0.0156					0.2500		0.0104			

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