Genetic Variability of Maryland and West Virginia Populations of the Federally Endangered Plant *Harperella nodosa* (Rose) (Apiaceae)

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Abstract - Riparian landscapes are dynamic systems and exhibit considerable spatio-temporal variation in stream flow and physical composition of stream substrates that provide habitats for many species. We investigated genetic diversity and population genetic structure of *Harperella nodosa* (Harperalla; Apiaceae), a federally endangered semi-aquatic plant. We employed a unique study design that involved sampling at regional, stream, and fine scales in 3 riverine systems in Maryland and West Virginia. Using intersimple sequence repeats (ISSRs), we found high levels of genetic diversity at all scales and pronounced fine-scale genetic structure. Pairwise correlation between geographic and genetic distance was scale-dependent. This study illustrates that temporal monitoring and multiple-scale plans are essential for conservation management programs for Harperella.

Introduction

Many rare plant populations consist of spatially discrete patches isolated various distances from other patches (Honnay et al. 2009). Geographic distance between patches can complicate assessments of demographic viability because isolated patches may or may not be connected by gene flow, and conservation biologists often do not have the information necessary to distinguish conservation or management units (Funk et al. 2012, Moritz 1994, Palsbøll et al. 2007). Additionally, when plants have the capacity for vegetative (clonal) growth, determining the number of individuals is often problematic, confounding one of the most basic parameters of demography. The assessment of potential gene flow among populations is complicated by the fact that realized dispersal may not conform to simple isolation by distance-dispersal models (Slatkin 1993) but may instead exhibit metapopulation dynamics (Hanski 1999) with complex dispersal patterns. Assessments of genetic diversity and the spatial arrangement of alleles using molecular markers can provide much-needed insight for conservation managers (Frankham et al. 2002).

Connectivity between populations is vital for genetic exchange and recolonization after local extinction. Riparian landscapes often experience high spatio-temporal heterogeneity in habitat availability and landscape connectivity (Lundqvist and Andersson 2001, Schleuning et al. 2011, Van Looy et al. 2009). In these dynamic habitats, plants appear, disappear, and reappear according to naturally occurring

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changes in stream flow and in response to physical reworking of stream substrates during periodic flood events. Agricultural practices, such as run-off from pollutants and irrigation drawdown, can further isolate populations within river systems and threaten ecosystem functioning (Kominoski et al. 2013, Tilman et al. 2002, Tscharntke et al. 2005). Thus, current land-use practice can create additional risks to small populations in dynamic riparian ecosystems.

Metapopulation theory provides a conceptual framework for understanding the nature of complex environmental heterogeneity and dispersal scenarios in riverine systems (He et al. 2004, Honnay et al. 2010, Markwith and Scanlon 2007, Schleuning et al. 2011). Genetic metapopulation structure mirrors population genetic processes and has temporal (Giles and Goudet 1997, Pierson et al. 2013), population-size (Frankham 1996), environmental (Manel et al. 2012, Schleuning et al. 2011), and reproductive components (Van Looy et al. 2011, Lundqvist and Andersson 2001). Ephemeral patches and recurrent local extinction are commonplace in plant populations along rivers, and genetic markers allow for reconstruction of current and past population changes.

Harperalla nodosa (Rose) (Harperella; Apiaceae) is a federally endangered semi-aquatic plant species native to rocky streams in Maryland, West Virginia, Virginia, North Carolina, Alabama, and Arkansas and non-riparian habitats in South Carolina Oklahoma, and Georgia (Buthod and Hoagland 2013, UFSWS 2008). In this study, we assessed genetic variability of this species and demonstrated the application of genetic tools in revealing genetic structure in Harperella. Specifically, we investigated the spatio-temporal structure of genetic variation within Harperella by assessing: (1) the genetic diversity within 3 regional populations, (2) differences in genetic diversity between 2 years (2009 and 2011), and (3) genetic structure at 3 geographic scales.

Materials and Methods

Study species

Harperella was listed as an endangered species in 1988 because of multiple types of habitat loss and declines in water quality (Bartgis and Maddox 1993). Twenty-six populations have been reported (Wells 2012a), with an additional population recently recorded from Oklahoma (Buthod and Hoagland 2013). The largest populations occur in West Virginia, Maryland, and Arkansas. Rose (1906, 1911) originally described 3 taxa in the genus *Harperella* (transferred to *Ptilimnium* by Mathias [1936]) based upon ecological discontinuities: *H nodosa*, a plant of pools and ponds; *H. fluviatilis*, a plant of rocky streams; and *H. viviparum*, also a plant of rocky streams but differing in the production of late-season vegetative ramets. Feist et al. (2012) redefined *Ptilimnium* on phylogenetic analyses of nuclear and chloroplast DNA corroborated by leaf morphology and fruit anatomy. They concluded that the genus *Harperella* was distinct from *Ptilimnium* and brought back *Harperella nodosa* (*P. nodosum* remains the name listed under the Federal Endangered Species Act; UFSWS 1988), but found no evidence to support the presence of the two other *Harperella* species described by Rose (1906, 1911). We use the common name Harperella throughout this manuscript.

Harperella exhibits a mixed mating system highly dependent upon insect-mediated pollination; most pollinations are expected to be geitnogamous due to weak interfloral protandry and vegetative spread (Marcinko and Randall 2008). Plants flower in July and August, releasing seeds in September and October, after which germination can occur immediately (Maddox and Bartgis 1992, Wells 2012b). The combination of selfing and asexual reproduction serves to increase short-term persistence at sites and enhance colonization ability. Microhabitat features also play an important role in colonization and persistence of Harperella (Frye and Tessel 2012, Marcinko and Randall 2008, Wells 2012a, Wells et al. 2004). The sexual life cycle of Harperella has been debated (USFWS 2008). To some extent the species acts as an annual, completing its life cycle in a single season; however, individual genets may be preserved via production of vegetative offshoots from the base and nodal buds. The parent rosette, vegetative shoots, and seedlings are all capable of overwintering, therefore raising questions as to whether this species acts more as a perennial than an annual.

Collections

Study locations. We sampled 3 streams within Maryland and West Virginia (Fig. 1). Sideling Hill Creek is a 40-km-long stream originating in Pennsylvania and flowing south to the Potomac River in Maryland. It contains a large population well-dispersed over approximately 10 stream km. According to a 2008 stream census, Sideling Hill Creek held approximately 47,262 flowering stems (D. Landau, pers. comm., The Nature Conservancy, Maryland/DC). Fifteen Mile Creek is a 31-km-long stream west of Sideling Hill Creek, also originating in Pennsylvania and flowing south into the Potomac River in Maryland, with its outflow approximately 6.8 stream km from that of Sideling Hill Creek. Fifteen Mile Creek contains a localized patch of 2–300 stems (<10 m^2) that has persisted at least since 1988. Sporadic occurrences of individual plants and small patches (2–100 stems) have been observed 0.1–4 km downstream but have not persisted. The final site, Sleepy Creek, is a 70-km-long stream that originates in Virginia, flowing north into West Virginia, before entering the Potomac River east of Sideling Hill Creek (Fig. 1). The outflow of Sleepy Creek is ~28 stream km from Sideling Hill Creek and ~34.8 stream km from Fifteen Mile Creek. Sleepy Creek also contains a large, well-dispersed population across ~32 stream km. According to a 2008 census, Sleepy Creek held approximately 400,000 plants, a substantial decline from an estimated 2 million in 1990 (USFWS 2008).

Water flow peaks in all 3 streams during winter and spring, but lies at less than full-bank during much of the late summer and fall when rocky shoals and bedrock are exposed. Habitat compositions of shales, siltstones, and fine-grained sandstones can all be found (MGS 1968). The extreme variance in demographic estimates is one of the most difficult problems in monitoring Harperella populations because the estimated population sizes vary not only due to annual reproduction and recruitment but also differ greatly among observers, census method, and the timing of the survey (Frye and Tessel 2012, USFWS 2008).

Sampling procedure. We sampled Harperella at 3 scales: regional, within-stream, and fine-scale plots. We collected 200 Harperella samples from the 3 streams for the regional analysis—2 in Maryland (Fifteen Mile Creek and Sideling Hill Creek) and 1 in West Virginia (Sleepy Creek)—and recorded latitude and longitude for each sampling location. Sideling Hill Creek was sampled in both 2009 and 2011, with a total of 142 individuals from 20 different sampling locations and 5–11 individuals per sampling location. In 2011, we also sampled 14 individuals from one sampling location at Fifteen Mile Creek and 44 individuals from 5 sampling locations along Sleepy Creek. We conducted fine-scale sampling at 2 streams: an upstream location in Sideling Hill Creek in 2009, a downstream location in Sideling Hill Creek in 2011, and at 1 location in Sleepy Creek in 2011. This fine-scale sampling consisted of intensive sampling from dense, localized patches to measure fine-scale genetic variability. We divided 1 rectangular plot (1.0 m^2) into 4 quadrants and took 4 samples from each of the 4 quadrants—one from each corner—for a total of 16 individuals from each plot.

ISSR Analysis

We extracted DNA from 0.02 g of tissue from each of 200 individuals using Qiagen DNeasy® extraction kits (Qiagen, Valencia, CA). We modified the extraction
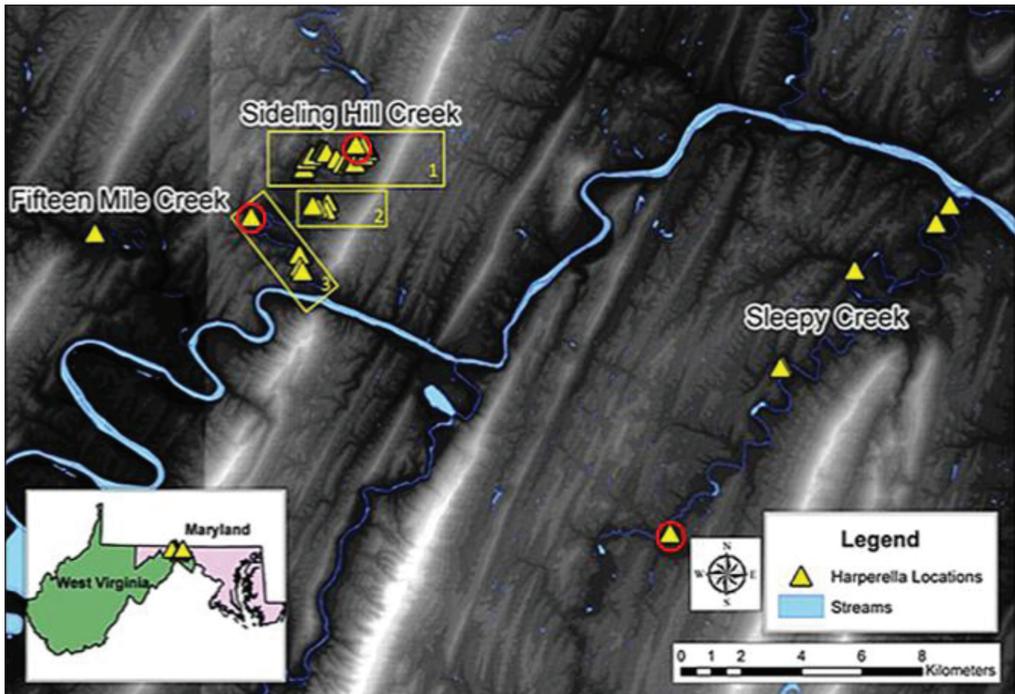

Figure 1. Sampling locations of 3 populations of Harperella in Maryland and West Virginia. There are 3 sampling scales within this project: (1) regional creek systems: Sideling Hill Creek (20 sites), Sleepy Creek (5 sites), and Fifteen Mile Creek (1 site); (2) 3 areas within Sideling Hill Creek (indicated by yellow rectangles); and (3) fine scale: 2 sites at Sideling Hill (13–16 individuals per site) and 1 at Sleepy Creek (16 individuals) (each fine-scale site indicated by a red circle around the yellow triangle).

technique in order to increase DNA concentration by adding sand to plant material and performing thorough grinding with a mini-micro pestle. Grinding time was positively correlated with final DNA concentration (K. Hunter, unpubl. data). We amplified DNA by means of intersimple sequence repeats (ISSR) using an Eppendorf autorisierter thermocycler with initial denaturation carried out for 1 min at 94 °C, followed by 35 cycles of 40 s at 94 °C, 45 s at 48–50 °C, 1 min 30 s at 72 °C, and a final 5-min extension at 72 °C. We scanned 15 ISSR primers from the University of British Columbia (UBC) primer set for detectable polymorphic banding patterns. Three primers produced consistent banding patterns: UBC 807, $[AG]_8T$; UBC 812, $[GA]_8A$; and UBC 841, $[GA]_8YC$. Amplifications were completed in a total volume of 25 µl consisting of 12.5 µl Promega GoTaq® (Promega Corporation, Madison, WI) colorless master mix, 10.5 µl deionized water, 1 µl primer, and 1 µl genomic DNA. These PCR reactions were then characterized on 1.5% agarose gels in 1X TBE buffer and stained with ethidium bromide. We ran replicate samples to check for reliability and reproducibility of the bands; band size was estimated from a 200-bp ladder (Promega). We visualized and analyzed ISSR bands using UV photography and Kodak 1D 3.6 Digital Analysis software, scoring data as presence or absence of bands.

Data analysis

Regional scale. We sampled 3 riverine systems for regional-scale comparison: Sideling Hill Creek, Fifteen Mile Creek, and Sleepy Creek. We calculated 3 measures of genetic diversity: the proportion of polymorphic loci (PPL), Nei's genetic diversity (H), and Shannon's information index (I), using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). A hierarchical analysis of molecular variance (AMOVA) with permutation test (n = 9999; GenAlEx) was used to compare the genetic variance from the 3 regional sites.

We examined genetic structure using all 200 sampled individuals across the 3 sites with STRUCTURE 2.3.4. We used the admixture model, where each individual can draw a fraction of its genome from a number of genetic clusters, and no prior information on population location (Pritchard et al. 2000). This analysis assumes that the populations are in Hardy-Weinberg equilibrium and that the markers are unlinked (Pritchard et al. 2000). We assumed a model of k populations (where k is unknown), and we tested k-values from 2 to 12. We used a Markov chain Monte Carlo (MCMC) algorithm to cluster all 200 individuals (burn-in = 10,000; MCMC = 100,000). STRUCTURE HARVESTER (Earl and vonHoldt 2011) was used to estimate the highest delta k, the best estimate for population number among all individuals (Evanno et al. 2005). We ran STRUCTURE and STRUCUTRE HARVESTER again using this k value in order to create a visual graphic of population structure among all 200 individuals (burn-in = 100,000; MCMC = 1,000,000).

Finally, we performed a Mantel test to evaluate evidence of isolation by distance by determining the relationship between pairwise genetic distance and geographic distance in km (GenAlEx 6.5; Peakall and Smouse 2006, 2012) using the latitude

and longitude for each collection site and performing a total of 9999 random permutations (Mantel 1967).

Within Sideling Hill Creek. We intensively sampled Sideling Hill Creek by sampling 141 individuals from this stream and, in order to identify smaller-scale genetic variation, partitioned these samples into 3 locations: upstream, gorge, and downstream. Indices of genetic diversity (PPL, H, and I) were estimated for each of these locations. To determine the within-site and among-site genetic variance of these locations, we conducted a hierarchical AMOVA with permutation test ($n =$ 9999; GenAlEx). To determine if there were differences by year, we also calculated genetic diversity by partitioning the data by collection year within Sideling Hill Creek: 2009 and 2011. We collected the majority of individuals from the upstream portion in 2009 and the downstream portion in 2011. In addition, we performed a Mantel test to investigate isolation by distance, following the same conditions as we used in the regional analysis.

Fine scale. The 45 samples we collected from each of the three 1.0-m^2 plots (Sideling Hill Creek in 2009, $n =13$; Sideling Hill Creek in 2011, $n = 16$; and Sleepy Creek in 2011, $n = 16$) were examined to represent fine-scale comparisons. We calculated genetic diversity values for each of the 3 fine-scale plots using the same protocol as previously described, and estimated population structure within each of these plots using STRUCTURE 2.3.4 with the same conditions as described above. An AMOVA was conducted to compare the genetic variability within and among the fine-scale sampling sites.

Results

Regional scale

We found a total of 42 unique polymorphic loci from the ISSR analysis among all 200 samples. Each stream exhibited high genetic diversity, with Fifteen Mile Creek and Sleepy Creek having higher levels of genetic diversity over the individual sampling year than Sideling Hill Creek (Table 1). The AMOVA results indicate that 80% of the genetic variation occurred within regional streams and 20% of the variation was found among the 3 riparian areas (Table 2). STRUCTURE estimated the highest delta $k = 3$. Population structure was seen throughout the regional analysis, although homogenous structure was detected among individuals at the same sampling locations (Fig. 2). This finding is indicated by the 3 colors present in all regional sites instead of only 1 color in each site; the genetic population clusters did not correspond to a stream system. However, there is little structure within sampling locations. There was no significant relationship between pairwise genetic distance and geographic distance when all populations were considered ($r_M = 0.075$, $P = 0.325$).

Within Sideling Hill Creek

We detected the highest values of genetic diversity downstream and the lowest values from samples collected in the gorge (Table 1); this result was most likely

W.B. Smith, C.T. Frye, E. Veliz, S. Hiebler, R.C. Taylor, and K.L. Hunter

attributable to the lower number of individuals within the gorge due to fewer available microhabitats. The AMOVA indicated significant genetic differentiation among the 3 locations—upstream, gorge, and downstream—sampled across both years combined (PhiPT = 0.164, $P < 0.0001$; Table 2). Sixteen percent of the total variation was found among the 3 locations; 84% of the total variation was attributed to within-site variation. We observed greater genetic diversity in samples collected in 2009 compared to 2011 samples (Table 1). However, the 2009 sample came from a much larger sampling area. There was a significant relationship between pairwise genetic distance and geographic distance in Sideling Hill Creek ($r_M = 0.266$, $P < 0.001$).

Table 1. Genetic diversity of Harperella from the 3 sampling scales: regional creeks, within Sideling Hill, and fine-scale sampling from Sideling Hill and Sleepy Creek. Measures of genetic diversity include: percent polymorphic loci (PPL), Nei's genetic diversity (H), and Shannon's information index (I). We omitted 3 samples from Sideling Hill Creek, MD (Upstream/2009) and 1 sample site was excluded from Sideling Hill.

Site	n (sample sites)	PPL	H	I
Regional scale				
Sideling Hill Creek, MD	112 (20)	95	0.378	0.552
2009	54(8)	43	0.160	0.238
2011	58(11)	22	0.083	0.124
Fifteen Mile Creek, MD	14(1)	69	0.208	0.321
Sleepy Creek, WV	28(5)	66	0.220	0.334
Within Sideling Hill				
Upstream	76(11)	95	0.360	0.531
Gorge	21(4)	69	0.233	0.351
Downstream	44(5)	92	0.358	0.525
Fine scale				
Sideling Hill Creek (upstream/2009)	13(1)	45	0.161	0.240
Sideling Hill Creek (downstream/2011)	16(1)	59	0.214	0.321
Sleepy Creek	16(1)	47	0.195	0.283

Table 2. Analysis of molecular variance for 200 individual Harperella plants from each sampling scale: regional, Sideling Hill, and fine-scale sampling from Sideling Hill and Sleepy Creek. *P*-value estimates are based on 9999 permutations.

AMOVA analysis	df	SS	MS	% variance	Φ - Statistic	*P*-Value
Regional scale						
Among creeks	2	117.686	58.843	20	0.204	<0.0001
Within creeks	129	722.397	5.600	80		
Within Sideling Hill						
Among plots	2	108.665	53.330	16	0.164	<0.0001
Within plots	112	803.196	7.171	84		
Fine-scale						
Among plots	2	83.078	41.509	37	0.366	<0.0001
Within plots	42	181.500	4.321	63		

W.B. Smith, C.T. Frye, E. Veliz, S. Hiebler, R.C. Taylor, and K.L. Hunter

Fine scale

Genetic diversity values for the 3 fine-scale sample plots were similar to those found in the larger-scale regional sampling area (Table 1). We observed pronounced population structure within the fine-scale plot at Sleepy Creek in comparison with the 2 fine-scale plots at Sideling Hill Creek (Fig. 3). STRUCTURE identified 3 genetic populations: 2 populations were identified within Sleepy Creek, 1 of them
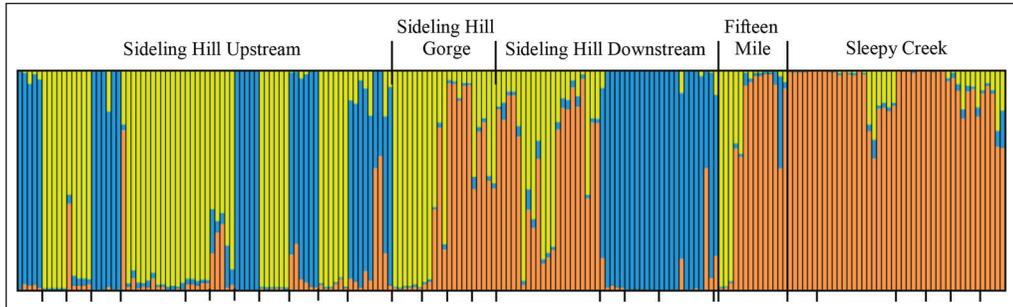

Figure 2. Population structure of 200 Harperella individuals sampled from 3 riverine systems estimated by the program STRUCTURE. The three colors represent three genetic populations ($k = 3$). Each individual is represented as a bar divided into color segments in proportion to the estimated ancestry in each of 3 clusters. Sampling locations include Sideling Hill Creek (partitioned to upstream, within the gorge (midstream), and downstream), Fifteen Mile Creek, and Sleepy Creek (top). Individual sites within the 3 sampled streams are separated by lines at the bottom of the graphic.
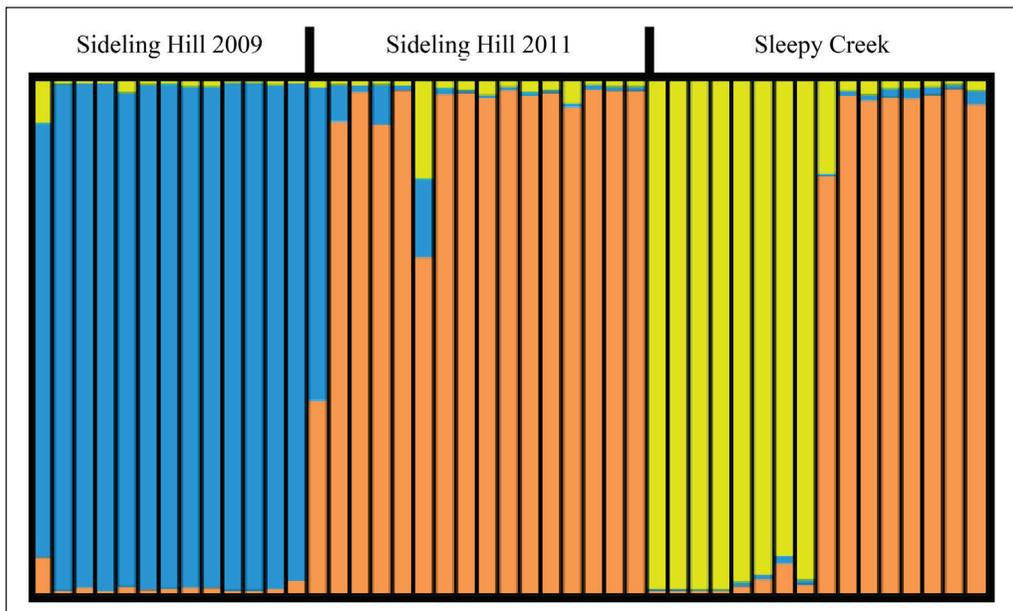

Figure 3. Fine-scale STRUCTURE analysis from three 1.0-m^2 plots: 2 sampling sites from Maryland (Sideling Hill Creek 2009 and 2011) and one from West Virginia (Sleepy Creek). This was a unique STRUCTURE analysis only analyzing the 3 fine-scale collections. Each bar represents an individual and the 3 colors indicate three genetic population clusters ($k = 3$).

grouped with Sideling Hill 2011, and a third population was identified in Sideling Hill 2009. The fine-scale genetic diversity values for Sideling Hill Creek were similar to those generated by 2009/2011 sampling, suggesting that we could recover most alleles at small scales. AMOVA results showed that 63% of the variation was found within fine-scale plots and 37% among plots (Table 2).

Discussion

Our research involved a study design featuring 200 samples of a federally endangered riparian plant species. Although Sideling Hill Creek in Maryland—sampled in 2 different years—was our main focus, we also had the opportunity to sample additional populations, 1 in Maryland and 1 in West Virginia. These sampling sites allowed us to analyze the molecular data at 3 different spatial scales—regional, stream-level, and fine scales. The regional analysis documented high levels of genetic variability in this endangered species at all 3 locations. The only previous molecular analysis of Harperella was done by Kress et al. (1994), and their allozyme study produced lower genetic diversity values in similar locations. Allozymes produce very different estimates of genetic diversity than ISSRs, and it is very difficult to compare diversity levels between different markers. Finger and Klank (2010) suggest allozymes as suitable for detection of genetic variation within and between populations, with the drawback of underestimating genetic variation.

The observed levels of genetic diversity and population structure may indicate remnants of high genetic variability and/or continuing gene flow between the stream systems. Population structure (Fig. 2) was documented in all 3 regional creek systems, but Sleepy Creek exhibited greater homogeneity. Pollination and seed dispersal have the ability to interact to contribute to overall gene-flow levels (Jordano 2010), although Richards et al. (1999) suggested that pollen-gene flow is only effective within several tens of meters. Wind dispersal may also play a role in gene flow in riparian landscapes, although the complexity of all these factors working together in the system is not yet well understood. Additionally, physical barriers within riparian landscapes, such as the gorge found within Sideling Hill Creek, may also alter gene flow by means of asymmetric dispersal.

Riparian habitats function very differently from other systems with regard to gene flow. The unidirectional diversity hypothesis (Markwith and Scanlon 2007, Ritland 1989) has been suggested to describe gene flow in a riparian landscape. This model states that seed dispersal occurs in the downstream direction, without upstream compensation, leading to an accumulation of genetic diversity downstream, with populations upstream becoming genetically impoverished (Honnay et al. 2010, Markwith and Scanlon 2007, Ritland 1989). This pattern is supported by our data at Sideling Hill Creek: Nei's genetic diversity was higher in the downstream segment despite our having roughly half of the number of sampling locations there as we had in the upstream segment (Fig. 3). This result is weakened somewhat because of the high variance within sampling sites at Sideling Hill (Table 2). We cannot eliminate the possibility that the observed differences simply reflect the sampling year. We cannot comment on upstream compensation of alleles via pollen because

our marker system cannot distinguish between the 2 sources of gene flow, i.e., seed versus pollen.

An alternative model for gene flow in riparian landscapes is the metapopulation model, which has been documented in riparian plant populations when colonization rates are able to offset local extinction (Hanski 1999, Honnay et al. 2009). Seed or pollen dispersal may happen in one of 2 ways: by dispersal between adjacent populations along the stream, with genetic distances between populations increasing with geographic distances, or by long-distance dispersal between non-adjacent populations, with an absence of isolation by distance and low genetic differentiation among populations (Honnay et al. 2010). Tero et al. (2003) suggest a classic metapopulation model in the endangered *Silene tatarica* L. (Tartarian Catchfly) based upon the lack of a relationship between pairwise genetic distance and geographic distance. We found a similar lack of relationship in Harperella at the largest scale (all 3 streams), implying gene flow and a regional gene pool. However, within Sideling Hill Creek, we found a significant relationship between pairwise genetic distance and geographic distance, inferring that there is isolation by distance, and migration is chiefly occurring within population segments (Jacquemyn et al. 2010). We attribute this result to the presence of a local geographic feature (the gorge) acting to restrict upstream gene flow in some years. We hypothesize that episodic gene flow occurs during very favorable years, which may include mild winters and extended periods of hydrologic drawdown when flowering rate is high. We suggest episodic rather than continual or stepping-stone models because when we examined variation both within and between population segments (Table 2) and overall genetic structure (Fig. 2), we concluded that a mix of both short- and long-distance dispersal is or has been occurring. This pattern was described by Markwith and Scanlon (2007) as a nonadjacent flow model, occurring in more of an asymmetrical pattern, which may serve as an appropriate hypothesis for our study system.

One of our most striking findings was that the small and very restricted population at Fifteen Mile Creek had levels of genetic diversity comparable to the much larger populations at Sideling Hill and Sleepy Creek (Table 1). We had assumed a priori that plants at this location would be one or a few persistent clones because this is an isolated location with episodic low numbers of individuals. In contrast to our expectations, the small patch of plants available at this stream exhibited comparable levels of genetic diversity and evidence of past gene flow and the presence of each of the 3 genetic clusters. We attribute the persistence of genetic diversity within this small population to vegetative propagation and conclude that this system acts to reduce the effects of genetic drift. Further, we may now conclude that environmental factors, and not genetic ones, are responsible for the observed small population and spatial restriction of Harperella in this stream.

Our finding of genetic structure and high genetic diversity in fine-scale plots is of practical and theoretical importance. It is of practical importance because estimates of population size have long been hampered by our ignorance of the number of genets in dense patches. These large patches (by some estimates comprising >1000 stems) may persist for decades and were suspected of being composed of

one or a few clones. We conclude that counting each stem as genet and not a putative ramet of a large clone is supported by the evidence. Levels of genetic diversity (Table 1) and population genetic structure (Fig. 3) in fine-scale plots mirrored those of stream-wide samples (Table 1 and Fig. 2). All 3 fine-scale plots indicated evidence of past gene flow because all 3 genetic clusters were identified in each plot (Fig. 3). This result suggests that we can recover most alleles present during any sampling year at very small scales and is of theoretical importance for developing sampling strategies for range-wide studies. We detected pronounced fine-scale structure in Sleepy Creek when compared to two plots in Sideling Hill Creek. This pattern suggests high sexual recruitment at Sleepy Creek versus more local seed dispersal or vegetative propagation in the Sideling Hill plots, and is consistent with expectations of samples from very large populations.

Harperella is an endangered plant that exists in a system that experiences extreme stochastic events. Continued reduction in Harperella population sizes could lead to genetic impoverishment, which is expected to further increase the extinction risk (Honnay et al. 2010). We suggest that without the linking populations along the Potomac River, each stream is now on its own evolutionary trajectory. Further, we conclude that the current absence of Harperella along the Potomac River has not had a dramatic effect on genetic diversity and population structure in this species. We hypothesize that the vegetative propagation of genotypes acts to preserve genetic diversity by slowing the effects of genetic drift. Therefore, questions need to be addressed concerning the best monitoring practices for the species' conservation. It is evident that temporal monitoring and multiple-scale plans are essential. We caution that managers should not be quick to assign management units to isolated streams without first considering temporal variance and historical connectivity. Honnay et al. (2009) found differences in genetic variation between years in a riparian species. Because this study found the potential for similar variation between years in Harperella, a sampling design should be carefully planned out. In order to clearly look at temporal variance in genetic diversity, the monitoring design should include samples collected throughout the range in multiple years. If possible, the protocol should employ complete genome sequences (Allendorf et al. 2010) and landscape genetic-analysis techniques that consider spatial differentiation as a product of multiple population forces acting over time (Manel and Holderegger 2013, Marko and Hart 2011). Agencies mandated to monitor and/or restore populations of endangered species, such as Harperella, must undertake a progressive conservation plan that links reproductive biology, population genetics, and population dynamics at local and regional scales. This type of coordinated management approach would yield a more integrated understanding of this complex system.

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