RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration
SER SOCIETY FOR ECOLOGICAL RESTORATION

RESEARCH ARTICLE

Riverine complexity and life history inform restoration in riparian environments in the southwestern United States

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Riparian habitat in the southwestern United States has undergone substantial degradation over the past century, prompting extensive management and restoration of these critical ecosystems. Most restoration efforts, however, do not account for life history traits or riverine complexity that may influence genetic diversity and structure. Here, we use simple sequence repeat markers in four southwestern riparian species (*Populus fremontii*, *Salix gooddingii*, *S. exigua*, and *Prosopis glandulosa*) that occupy a geographically complex region to address four questions: (1) How is river connectivity related to genetic diversity and structure? (2) How do mating systems and dispersal mechanisms influence gene flow? (3) Is genetic diversity influenced by unidirectional water flow? (4) How do unregulated tributary and regulated river flows affect clonality and associated diversity? Our results identify five findings: (1) Patterns of genetic diversity and structure vary substantially across different species; (2) species with geographic distributions that include a large, perennial river exhibit the least genetic structure; (3) mating system, clonality, and seed dispersal are related to genetic structure; (4) genetic diversity is variable among species and populations, but does not increase or decrease unidirectionally; and (5) clonality and associated diversity does not differ along a regulated river relative to unregulated tributaries. Our multispecies approach to understanding how riverine complexity and life history traits influence genetic diversity and structure could be incorporated into management efforts to more closely match riparian species with their unique environments, thereby facilitating restoration success.

Key words: genetic diversity, genetic structure, Grand Canyon, native plant material, riparian plants, riparian restoration

Implications for Practice

- Genetic diversity, structure, and life history of riparian plant species are critical considerations for riparian restoration, as these vary across species and diverse river systems.
- When using multiple plant species in restoration, different species will likely require different management choices. Managers can incorporate differences by considering dispersal mechanisms and river system complexities in decisions regarding genetic diversity maintenance, transfer distances, coevolved communities, and maintaining connectivity.
- Since riparian ecosystems are threatened worldwide, it is imperative that restoration efforts account for the factors that maintain evolutionary potential. Maintaining genetic diversity of riparian forests through identifying high-diversity sites plays an important role in this effort by highlighting key areas for conservation and potential source locations for plant propagation.

Introduction

Genetic variation and structure can influence a species' ability to adapt to changing climate (Parmesan 2006; O'Neill et al. 2008), alter evolutionary resilience (Sgrò et al. 2011), and ultimately affect population viability (Thomas et al. 2004). Genetic variation, which is key to adaptation, is often influenced by factors that arise from geographic complexity or landscape connectivity (e.g. rivers, mountains, canyons), mating system (e.g. outcrossing, inbreeding), environmental variation

Author contributions: ECP, GJA, KO, TGW, BJB conceived and designed the research; ECP performed the field and genetic work; ECP, GJA, KO analyzed the data; TGW, BJB, PBS contributed materials and analysis tools; ECP, GJA wrote the manuscript; all authors edited the manuscript.

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doi: 10.1111/rec.13418
Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.13418/suppinfo

(e.g. temperature, precipitation), and life history traits (e.g. seed dispersal, longevity) (Hamrick et al. 1992; Manel et al. 2003). Increasingly, evolutionary resilience and local adaptation are being incorporated into restoration efforts by characterizing genetic variation and structure of the species of interest (Sgrò et al. 2011; Hoban et al. 2013), which then guides selection of native plant materials (e.g. Durka et al. 2017; Massatti et al. 2020) including the restoration and management of riparian areas (Bothwell et al. 2017; Ikeda et al. 2017).

Vegetation restoration along river corridors (hereafter, "riparian restoration") is a global undertaking (González et al. 2015) and can benefit from the integration of genetic information. Understanding genetic diversity and structure of source populations is particularly important, because the use of stem cuttings and root stock propagation is a common practice (Del Tánago et al. 2012; González et al. 2015; Ralston & Sarr 2017). Such practice, however, can result in reduced allelic and genotypic diversity that can remain on the landscape for many years, even for obligate outcrossing, wind-dispersed species (Lin et al. 2009). Efforts to incorporate genetic variation and structure into riparian restoration have largely focused on individual species (e.g. Smulders et al. 2008; Mosner et al. 2012; Eusemann et al. 2013). Restoration of riparian areas, however, usually includes multiple plant species, which may exhibit different genetic patterns given unique topographic habitat characteristics and life history traits.

Unique geographic patterns, life history traits, and environmental pressures differentially affect gene flow and associated patterns of genetic variation in riparian plants (Morrissey & de Kerckhove 2009; Honnay et al. 2010; Paz-Vinas et al. 2015). River systems are both dendritic and linear (Morrissey & de Kerckhove 2009), controlled by hierarchical physical processes (Montgomery 1999), driven by variable flow patterns (Lytle & Poff 2004), and are conducive to asymmetric transport of propagules, pollen, and other resources in a downstream direction (Paz-Vinas et al. 2015). Genetic variation in riparian plants can be closely related to abiotic characteristics of riparian areas, such as watersheds (Lin et al. 2009; Hernández-Leal et al. 2019), river connectivity and size (Cushman et al. 2014; Werth et al. 2014), flow variability and flood frequency (Douhovnikoff et al. 2005; Pollux et al. 2007), and temperature and precipitation variability (Bothwell et al. 2017). It can also be related to biotic characteristics, such as dispersal (Love et al. 2013), clonal growth (Douhovnikoff et al. 2005; Pollux et al. 2007), and vegetation type and fragmentation (Hoply & Byrne 2018). Asymmetrical gene flow in dendritic systems can lead to higher genetic diversity in downstream reaches or high regional diversity due to differentiated headwater populations (Morrissey & de Kerckhove 2009; Paz-Vinas et al. 2015). Changes to river flows, particularly stabilization of base flows, can increase clonal growth and alter patterns of diversity and stand structure (Douhovnikoff et al. 2005; Pollux et al. 2007; Smulders et al. 2008; Eusemann et al. 2013). Stabilized flows reduce disturbance and regeneration from seed, both of which can lead to greater clone size and dominance (Douhovnikoff et al. 2005; Pollux et al. 2007; Smulders et al. 2008; Eusemann et al. 2013). These factors can be generalized into mechanisms related to river size, life history traits, climate, and the strength of asymmetrical gene flow. For restoration practitioners, this can have important ramifications for transferring plant material among rivers, prioritizing upstream or downstream habitats, or planning for changes in climate and river connectivity. Furthermore, depending on the distributions and life history traits of the species being used for restoration, these mechanisms may result in coexisting species with different genetic patterns, which may require different restoration strategies.

Examining relationships between properties of riparian systems and riparian plant genetic patterns for multiple species across a highly variable region can better inform riparian restoration. Wind pollination and water dispersal should result in extensive gene flow across broad areas, but insect pollination, decreasing river size, land barriers, and differences in precipitation and temperature (Hamrick et al. 1992; Cushman et al. 2014; Bothwell et al. 2017) can restrict gene flow with concomitant increases in genetic structure. River regulation can increase clonal growth, impacting genetic patterns (Pollux et al. 2007; Smulders et al. 2008; Eusemann et al. 2013). Gaining a better understanding of these relationships can help guide native plant restoration (Massatti et al. 2020).

The Grand Canyon region of northern Arizona, U.S.A. (Fig. 1), is an environmentally diverse area suitable for examining patterns of genetic structure and diversity in riparian plant species. This region is dissected by large canyons, dry terrain, complex geography, and steep elevation and climate gradients (Stortz et al. 2018). The dam-regulated Colorado River, one of the largest, perennial rivers in the southwestern United States, both divides and connects broad environmental gradients and a large geographic area (approximately 2 million hectares). River connectivity, in the sense that large rivers facilitate gene flow and small and intermittent streams inhibit gene flow (Cushman et al. 2014), varies from the highly connected Colorado River to disconnected, small-volume intermittent and perennial tributaries (Stortz et al. 2018). Differences in riparian plant geographic distributions across this landscape can be leveraged to better understand genetic patterns important to restoration.

Here, we focus on four native riparian plant species commonly used in revegetation in the southwestern United States: *Populus fremontii* S. Watson (cottonwood), *Salix gooddingii* C.R. Ball (Goodding's willow), *Salix exigua* Nutt. (coyote willow), and *Prosopis glandulosa* Torr. (honey mesquite). Species within *Populus* and *Salix* are foundation species in many riparian systems and are regularly used in riparian restoration globally (Whitham et al. 2006; González et al. 2018). Species in the genus *Prosopis* have been used for restoration due to their many ecological and cultural uses and are also of interest because some are problematic invasive taxa (Shackleton et al. 2014). These four species are common in the southwestern United States, but differ in their life history traits and distribution (Table 1).

To examine riparian plant genetic patterns in the context of restoration, we address four hypotheses: (1) Riparian plant species that are patchily distributed across low flow volume tributaries (less river connectivity) will exhibit more genetic structure and differentiation than species whose distribution includes a large river (more connectivity). (2) The degree of

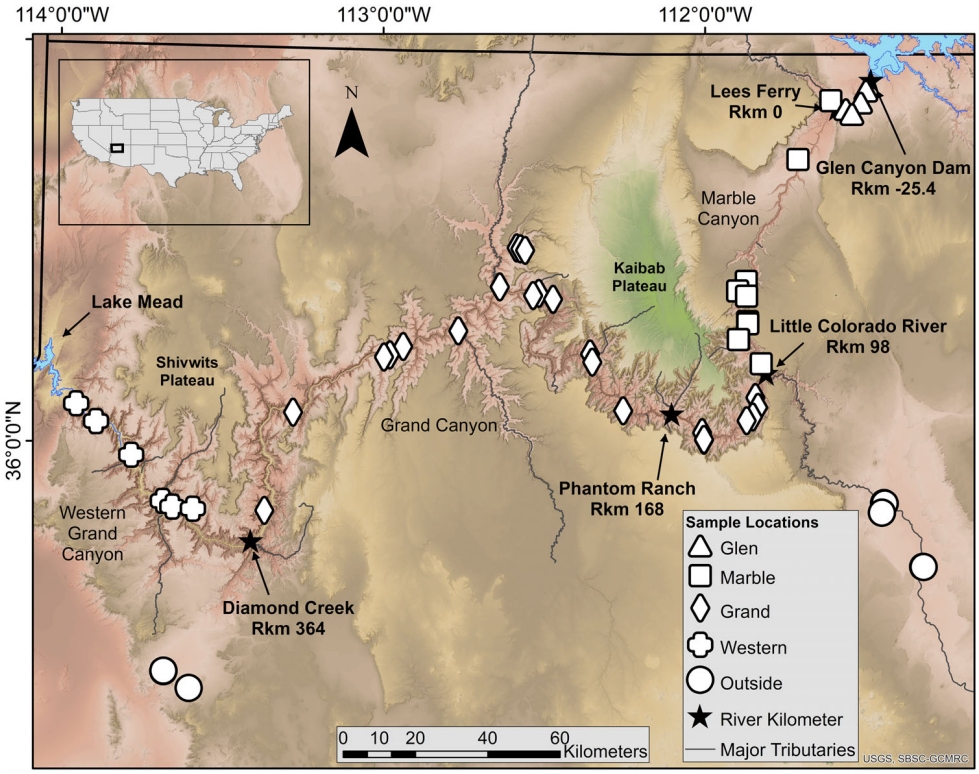

Figure 1. Map of the course of the Colorado River through Glen, Marble, and Grand canyons in northern Arizona, U.S.A., and all sample locations. Water flows east to west from Glen Canyon Dam to Lake Mead. Samples were collected along the Colorado River and in tributaries. Not all species occurred at each location. Notable landmarks and their distance from Lees Ferry, Arizona, along the Colorado River are shown in kilometers (river kilometer, Rkm). Segments that defined four of the five study subregions are as follows: Glen Canyon = Glen Canyon dam to Lees Ferry, Marble Canyon = Lees Ferry to Little Colorado River, Grand Canyon = Little Colorado River to Diamond Creek, western Grand Canyon = Diamond Creek to Lake Mead. Basemap colors indicate elevation with green for high elevation (up to 3,800 m), yellows for mid elevations, and pink for low elevation (150 m).

Table 1. Species used in this study, their relevant life history traits, and sample size (*N*). "Tributaries" refers to any ephemeral, intermittent, or perennial stream that empties into the Colorado River. *N* (sites, ind) = number of sites and individuals sampled.

Species	*Family*	*Growth Form*	*Distribution in Study Area*	*Pollination*	*Seed Dispersal*	*Mating System*	*Clonal*	N *(Sites, ind)*
P. fremontii	Salicaceae	Tree	Tributaries only	Wind	Wind/ Water	Obligate outcrosser	No	13, 191
S. gooddingii	Salicaceae	Tree	Tributaries and less frequently on Colorado River	Insect	Wind/ Water	Obligate outcrosser	No	10, 139
P. glandulosa	Fabaceae	Tree	Common on Colorado River, less frequent in tributaries	Insect	Animal/ Flood	Selfing possible	No	16, 225
S. exigua	Salicaceae	Shrub	Tributaries and Colorado River, common	Insect	Wind/Water	Obligate outcrosser	Yes	21, 308

genetic differentiation will be related to species' life history traits, such that obligate outcrossing, wind-pollinated, water-dispersed species will exhibit less structure than insect-pollinated, animal-dispersed species and/or clonal species. (3) Genetic diversity will be affected by geographic position, where genetic diversity is greater in downstream populations. (4) Clonality within a species will increase and diversity decrease along a regulated river relative to unregulated tributaries. We evaluate these hypotheses by determining the most likely number of genetic groups occurring in the region by species, comparing how differentiated those groups are within each species, and examining patterns of diversity and clonality across the region.

Table 2. Elevation range of collection sites within each subregion of the study area (site elevation range) and the collection sites that are included in each. Elevation ranges are separated into sites on the Colorado River (CR) and those in tributaries (T). Collection site codes under each species name follow codes in Figure 2 and Supplement S1. Site labels indicate geographic locations for *Populus fremontii*—O = outside, M = Marble Canyon, G = Grand Canyon; for all other species O = outside, M = mainstem of the Colorado River, T = tributaries to the Colorado River. Note that *P. fremontii* is in tributaries only.

Subregion	*Site Elevation Range (m)*	P. fremontii *Sites*	Salix gooddingii *Sites*	Prosopis glandulosa *Sites*	Salix exigua *Sites*
Outside	T: 1,245–1,435 m	O1, O2, O3	O1, O2	O1, O2	O1, O2, O3
Glen Canyon	CR: 945–970 m	None	None	None	M1–M4
Marble Canyon	CR: 845–880 m T: 965–1,055 m	M1–M3	None	M1–M3, T1	M5, M6, T1–T3
Grand Canyon	CR: 450–820 m T: 495–1,350 m	G1, G2–G6	M1, T1, T2, T3	M4–M9, T2	M7–M10, T4–T6
Western Grand Canyon	CR: 365–370 m T: 360–470 m	None	M2, M3, T4, T5	M10, T3, T4	M11, T7

Methods

Study Area

This study was conducted along the Colorado River and its tributaries within the Grand Canyon region in northern Arizona, U.S.A. (Fig. 1, Table 2). The Colorado River differs greatly from its tributaries in flow volume (much larger), permanence (perennial), and variability (hydroelectric dam regulated). Regulation by Glen Canyon Dam began in 1963; base flows range from 226 to 707 m^3/second, seasonal floods are greatly reduced, and base flows are higher and more stable compared to pre-dam flows (Gloss et al. 2005). Tributaries that support riparian vegetation are low volume or intermittent and have natural flow regimes (Stortz et al. 2018). Only five perennial streams flow into the Colorado River from outside the distinctive rim that creates Grand Canyon. Other tributaries start within Grand Canyon in their own canyons. Cushman et al. (2014) showed that gene flow in *Populus* diminishes as a function of river size, suggesting that species that occupy river networks will have greater connectivity along large rivers as compared to smaller rivers.

While typified by a generally warm and dry climate, temperature and precipitation vary dramatically across the plateaus and canyons, depending on elevation and topography. Collection site elevations ranged from 360 m to 1,435 m (Table 2). Along the 450 km segment from Lees Ferry, AZ, to Lake Mead (Fig. 1), the Colorado River drops in elevation from 950 m to 360 m, mean annual temperature increases by approximately 3.5°C, and precipitation timing shifts from summer- to winter-dominated (Caster & Sankey 2016). These shifts in climate are correlated with a shift in riparian species composition (Palmquist et al. 2018). Nonriparian, upland areas adjacent to our study sites are characterized by desert scrub and desert grasslands (Stortz et al. 2018).

Sample sites were within five broad subregions of the overall study area that correspond with key landscape features (Fig. 1, Supplement S1). The "Outside" subregion includes all tributary sites that are on the plateaus above canyons that characterize the region (Fig. 1, Table 2). These sites have low to no rock walls bounding the stream corridor. From these plateaus, the tributaries travel through deep canyons until joining the Colorado River. The other four subregions delineate areas that occur within canyons, below the plateaus, and include the Colorado River. Boundaries of these regions are given by points along the Colorado River, and encompass the canyons of the tributaries that join the Colorado River within that segment. The Kaibab Plateau changes the course of the river and weather patterns (Caster & Sankey 2016). In western Grand Canyon, large sediment deposits from Lake Mead dominate the shorelines. Where possible, multiple species were collected in the same location. Overlapping sample sites are noted in Table S1.

Study Species Distribution and Connectivity

We focus on four broadly distributed woody species commonly used in restoration in the southwestern United States. *Populus fremontii* is a dioecious, obligate outcrossing, wind-pollinated, and wind- and water-dispersed tree (Table 1). Stands of this species do not occur along the Colorado River in this region and are only found in tributaries. Of the four species, this species is the least connected by a large river. *Salix gooddingii* and *Salix exigua* are dioecious, obligate outcrossing, wind- and water-dispersed species pollinated primarily by insects. The former is a tall tree, while the latter is a moderate sized shrub. Stands of *Salix gooddingii* are generally found in tributaries, except for one stand in Grand Canyon and many in western Grand Canyon, so are more connected by a large river than *P. fremontii*. *Salix exigua* is common along the Colorado River and in tributaries. *Prosopis glandulosa* is a largely outcrossing tree or shrub, but can be self-compatible (Lopez-Portillo et al. 1993); it is pollinated by insects, and seeds are dispersed by animals (e.g. rodents, ungulates) and floods. It is common on the Colorado River and uncommon in tributaries. Stands of *S. exigua* and *P. glandulosa* are more connected by a large river than the other two species. *Salix exigua* is the only clonal species and can form large, genetically identical stands (Douhovnikoff et al. 2005).

Genetics

Sample sites for genetic material were chosen to represent the geographic distribution of each species within the Grand Canyon region (Table 1). Leaf tissue was collected from 15 individuals at each site, or every individual if there were fewer than

15 individuals (voucher collections listed in Table S1). For *P. fremontii*, *S. gooddingii*, and *P. glandulosa*, total genomic DNA was extracted from dried leaf tissue using a high-molecular weight protocol (Mayjonade et al. 2016) with minor modifications. For *S. exigua*, DNeasy Plant Minikits were used. After screening a selection of simple sequence repeat (SSR) loci for amplification, variability, and repeatable scoring, we amplified 9, 9, 11, and 13 loci for *P. fremontii* (Bothwell et al. 2017), *S. gooddingii*, *S. exigua* (Barker et al. 2003; Lauron-Moreau et al. 2013; Bozzi et al. 2015), and *P. glandulosa* (Mottura et al. 2005; Bessega et al. 2013), respectively. All loci and associated fragment analysis were processed on an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.) using GeneScan LIZ500 internal size standard (ABI). Allele fragment sizes were scored using GeneMarker v2.2.0 (SoftGenetics, LLC, State College, PA, U.S.A.).

Loci missing more than 5% of values, were not polymorphic, or could not be reliably scored were omitted. Frequency of null alleles was estimated using a Bayesian Individual Inbreeding Model (IIM) implemented in INEST v. 2.2 (Chybicki & Burczyk 2008, Supplement S2). Data for nine loci were used for *P. fremontii*, six for *S. gooddingii*, eight for *P. glandulosa*, and nine for *S. exigua* (Table S2).

For analyses of genetic structure, clone-correction was implemented for *S. exigua* (*poppr*, Kamvar et al. 2014, R version 3.6.1), reducing the number of individuals per site to only those that have unique multilocus genotypes (MLG). Five sample sites that had less than five MLG remaining were removed, resulting in the inclusion of 16 sites and 155 MLGs for genetic structure analyses. The clone-corrected dataset was used for all analyses other than diversity and clonality statistics. Data generated during this study are available from the USGS ScienceBase-Catalog (Palmquist & Allan 2021).

Assessment of Genetic Structure

We followed a standard practice of using multiple methods to infer genetic groups, since clustering methods have different known strengths and weaknesses (Janes et al. 2017; Miller et al. 2020). These included: (1) STRUCTURE version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003) evaluated using the Evanno et al. (2005) method (ΔK), (2) neighbor-joining (NJ) trees (*poppr*, R 3.6.1), and (3) *k*-means clustering (find.clusters, adegenet; Jombart 2008, R 3.6.1). The combined inferences of these methods provide a clearer illustration of genetic structure than using only a single method. We focus on results derived from STRUCTURE. Additional details on the other two methods are provided in the Supplement S3 and Table S3. For STRUCTURE, we used the admixture model with a burn-in of 20,000 followed by 200,000 iterations. This was repeated 20 times for each K (number of genetic groups) from 1 to the number of sample sites for each species. ΔK was implemented in Structure Harvester (Earl & vonHoldt 2012). For species with strong genetic structure, subsets of populations were evaluated for substructure using the same methods. As the ΔK method does not include $K = 1$ as a possible solution, admixture results, low bootstrap support in the NJ-tree analysis, and analysis of molecular variance (AMOVA) results were evaluated as indications of low to no structure among groups, implying $K = 1$. Admixture was assessed as function of membership probabilities. Replicate runs (20) were aligned and summarized using CLUMPP (Jakobsson & Rosenberg 2007).

Genetic Differentiation

AMOVA was run with 9,999 permutations to evaluate the distribution of genetic variation among individuals, sample sites, and genetic groups identified by STRUCTURE (poppr.amova in poppr, R). F_{ST}, a measure of population differentiation ranging from 0 to 1 where values close to 1 indicate high differentiation, was calculated (wc, hierfstat, Goudet 2005; R) and pairwise F_{ST} among sites was calculated within species (pairwise.WCfst, hierfstat; R).

Genetic Diversity and Clonality

Diversity statistics (number of private alleles, Simpson's diversity index, evenness, observed and expected number of multilocus genotypes, expected heterozygosity) were calculated for all collection sites and for NJ genetic groups with support >60 b.s. (poppr, private_alleles in poppr, R). Correlation between geographic position along the river and genetic diversity was quantified using Pearson's correlation coefficients between the river kilometer distance of each site from Lake Mead and expected heterozygosity (rcorr in Hmisc, R). A negative correlation indicates greater downstream genetic diversity. Separate correlation tests were run for each species using all sites and using only Colorado River sites for *P. glandulosa* ($n = 10$) and *S. exigua* ($n = 11$). The other two species had too few Colorado River sites (*P. fremontii*, $n = 0$; *S. gooddingii*, $n = 3$).

Differences in clonality and diversity in relation to regulated and unregulated flows were tested using *S. exigua* by comparing Colorado River ($n = 11$) and tributary ($n = 10$) sites. Clonality was calculated for each site as 1 – ([number of MLG – 1]/[number of samples – 1]), which varies from 0 (all individuals share an MLG) to 1 (all individuals have different MLGs) (Eusemann et al. 2013). Welch two-sample t tests were run (t.test, R) for clonality and expected heterozygosity.

Results

Genetic Patterns Vary by River Connectivity

The first hypothesis was supported in that genetic structure is not consistent across species and is in line with differences in river connectivity. We found that *Populus fremontii* (least connected) exhibited the greatest genetic structure, while the other three species showed little genetic structure. For *P. fremontii* (tributaries only), STRUCTURE identified $K = 2$ as the most probable number of genetic groups when all collection sites were included (Fig. S1), with sites outside of the canyons differing from those inside (Fig. 2A). When only sites inside the canyons were included, $K = 3$ was identified as the most probable number, with $K = 4$ slightly less probable (Figs. 2A & S2). The three groups exhibited little admixture and corresponded with Marble

Canyon, Grand Canyon, and one unique site at the junction of these subregions. With four groups, two sites in Grand Canyon formed a separate group admixing with Grand Canyon sites (not shown). In the AMOVA, 22.1, 27.7, and 50.2% of the total genetic variation was explained by differences among STRUCTURE groups, sites, and individuals, respectively (Table S4, all $p < 0.05$). *Populus fremontii* had F_{ST} of 0.31 and 17 private alleles based on genetic groups (Tables S5–S8).

For *Salix gooddingii* (sparsely distributed along the Colorado River), $K = 4$ was the most probable number of genetic groups (Fig. S3) with extensive admixture (Fig. 2B). In the AMOVA, 12.5, 11.0, and 76.5% of the total genetic variation was explained by differences among STRUCTURE groups, sites, and individuals, respectively (Table S4, all $p < 0.05$). *Salix gooddingii* had F_{ST} of 0.14 and 10 private alleles based on sites (Tables S5–S7, S9).

For *Prosopis glandulosa* (distributed extensively along the Colorado River), $K = 2$ was the most probable number of genetic groups (Fig. S4). Individuals belonging to each group were mixed within collection sites with no clear pattern related to geography (Fig. 2C). Both STRUCTURE and NJ tree analyses (Figs. S6 & S7) indicated two genetic groups widely spread across the region. The AMOVA resulted in 14.8, 22.7, and 62.6% of the total genetic variation explained by differences among STRUCTURE groups, sites, and individuals, respectively (Table S4, all $p < 0.05$). This species had F_{ST} of 0.20 and eight private alleles based on sites (Tables S5–S7 & S10).

For the widely distributed *Salix exigua*, $K = 2$ was the most probable number of genetic groups (Fig. S5). In the AMOVA, little (2.5%, $p = 0.08$) genetic variation was explained by differences among STRUCTURE groups and much more by sites (30.5%, $p = 0.00$) and samples (67.0%, $p = 0.00$, Table S4). Extensive admixture, the AMOVA results, and NJ-tree analyses all suggested that one widely distributed genetic group ($K = 1$) was more likely than $K = 2$ (Figs. 2D & S6; Tables S3 & S4). Pairwise differentiation among sites was high (Table S11), F_{ST} was 0.21, and 14 private alleles were detected (Tables S5–S7).

Genetic Patterns Are Differentially Related to Life History Traits

In contrast to our second hypothesis, the species with life history traits most likely to decrease genetic structure, *P. fremontii*, was the most genetically structured. The obligate outcrossing, wind pollinated, water dispersed, nonclonal species, *P. fremontii*, exhibited more genetic structure than the other species (Figs. 2 & S7; Tables S4 & S5). While exhibiting little overall genetic structure, the insect-pollinated, clonal *S. exigua* had significant site-level differentiation (Tables S4, S5, & S11) with many private alleles (14). The self-compatible, insect-pollinated, animal-dispersed species *P. glandulosa* showed significant site-level differentiation (Tables S4 & S10), but genetic groups were distributed across sites (Figs. 2C & S7).

Genetic Diversity Is Not Related to Position Along the River

Our third hypothesis was not supported in that genetic diversity was not found to increase as a function of distance downstream. The Pearson correlation coefficient between expected heterozygosity and distance from Lake Mead (COR_{He}) using all sites was $r = 0.46$, -0.55, -0.11, and 0.23 (all $p > 0.05$, Table S6), for *P. fremontii*, *S. gooddingii*, *P. glandulosa*, and *S. exigua*, respectively. COR_{He} for mainstem sites only was $r = -0.03$, 0.48 (all $p > 0.05$, Table S6) for *P. glandulosa* and *S. exigua*. For all species, diversity varied across collection sites (Tables S12 & S13). *Populus fremontii* had Simpson's genetic diversity of 0.99, expected heterozygosity (H_e) of 0.63, and three sites with notably low H_e (O3 = 0.22, G1 = 0.28, G6 = 0.36; Table S6). For *Salix gooddingii*, Simpson's diversity = 0.99, $H_e = 0.31$, one site had very low H_e (O2 = 0.19) and two sites had comparatively high H_e (M2 = 0.34, T5 = 0.36; Tables S6 & S12). For *P. glandulosa*, overall diversity = 0.99, $H_e = 0.55$, and a small population (M8) had very low H_e (0.16; Tables S6 & S12). For *S. exigua*, Simpson's diversity = 0.99, $H_e = 0.35$, and H_e is very low at sites with few MLG (Tables S6 & S12).

Diversity and Clonality Are Not Related to Unregulated Tributaries Versus Regulated River Flows

Our fourth hypothesis was not supported; clonality in *S. exigua* is not higher and diversity not lower along a regulated river relative to unregulated tributaries. Clonality between Colorado River and tributary sites was 0.48 versus 0.41 ($p = 0.48$). H_e did not differ between the two groups (0.24 vs. 0.27, $p = 0.33$). *Salix exigua* has many fewer MLG than individuals sampled (171 MLG vs. 308 individuals, 56%, Table S6). Two sites that occur along the same tributary share an MLG (O1, O2). Six sample sites have fewer than five MLG (Table S12).

Discussion

This study illustrates that riparian plant species within the same geographic region do not necessarily exhibit similar genetic patterns. This finding necessitates different management decisions regarding transferring plant material, prioritizing habitats, and planning for environmental change.

River Connectivity and Genetic Structure

Large, perennial rivers can facilitate gene flow across broad, environmentally heterogeneous landscapes, potentially influencing genetic structure and associated diversifying factors. Smaller tributary rivers appear to restrict gene flow. *Populus fremontii*, which only occurs in tributaries with low volume or intermittent surface flows, exhibits substantial genetic structure, despite being an obligate outcrossing, wind-pollinated, and wind- and water-dispersed species. In contrast, species that occur along the Colorado River (i.e. *S. gooddingii*, *P. glandulosa*, and *S. exigua*) show little genetic structure over more than 500 km of river. River size can influence genetic connectivity across the range of *P. fremontii* (Cushman et al. 2014), and this study suggests that this constraint is also relevant at a relatively small scale. Genetic differentiation is higher among Grand Canyon sites ($F_{ST} = 0.31$) than previously reported for the range of *P. fremontii* ($F_{ST} = 0.22$) based on Cushman et al. (2014). One

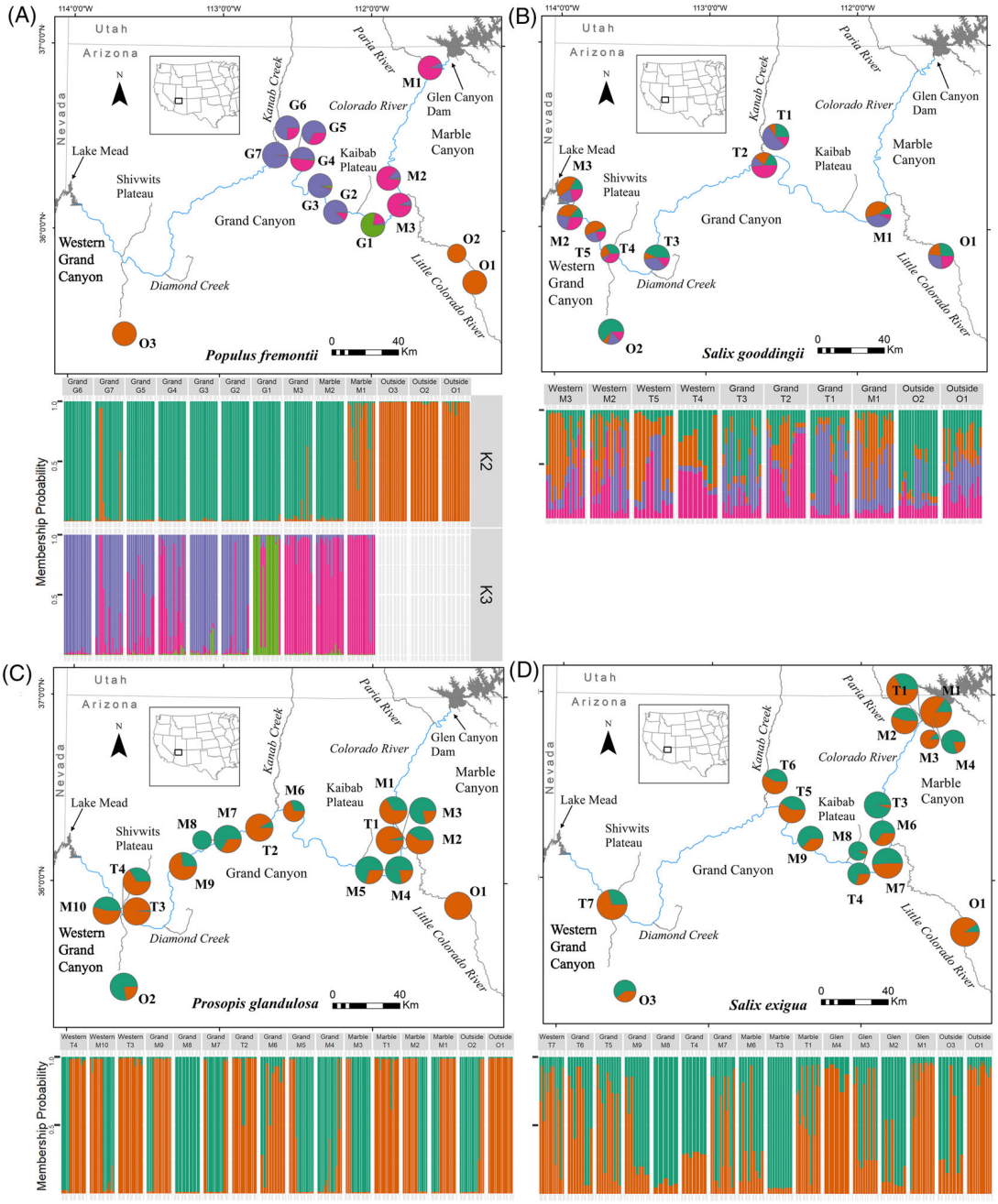

Figure 2. Maps and bar plots illustrating genetic structure based on STRUCTURE results. Site labels indicate geographic locations for (A) *Populus fremontii*—O = outside, M = Marble Canyon, G = Grand Canyon; for all other species (B–D) O = outside, M = mainstem of the Colorado River, T = tributaries to the Colorado River. The color proportions in each vertical bar (bar charts) or pie (pie chart) indicate the membership probability for each group. Symbol size on maps indicates number of samples. In bar plots, individuals are sorted by geographic region (top label) and collection site (bottom label). Collection sites are arranged from downstream (west) to upstream (east) to sites outside of the canyons. For (A) *P. fremontii*, results are shown for analyses that used all sites and two genetic groups ($K = 2$) and used only canyon sites and three genetic groups ($K = 3$). On the (A) *P. fremontii* map, genetic composition for outside sites use membership probabilities derived from $K = 2$, while the canyon sites use membership probabilities derived from $K = 3$.

possible explanation for this difference is the canyon topography and the lack of connection to riparian areas outside of Grand Canyon that result in more isolated populations. The large genetic differences exhibited between sites occurring outside of the canyons and those inside emphasizes the need to examine genetic substructure. Without separately analyzing the within-canyon sites, the genetic structure among them was not apparent. Even when focusing on within-canyon sites, further substructure may be of management interest here. When STRUCTURE analyses are conducted using four groups instead of three, two sites near each other within Grand Canyon (G4, G5) form the fourth group. The area comprising these two sites

contains archeological, agricultural features and an ancient cultivar of another plant species (Hodgson 2001). Understanding the context of substructure could lead to different management choices on whether or not to move plant materials to and from these locations.

Collection location choices for riparian species will differ depending on the management goals and the differences in genetic structure. For example, if the goal is to maintain existing genetic structure, plant material collection for less connected species, like *P. fremontii*, will be more restricted than for more connected species. This would mean not using plant materials from outside of Grand Canyon at sites within Grand Canyon and using materials developed from Marble Canyon and Grand Canyon in each of those respective areas. For species occurring along large, perennial rivers, restoration stock could include sources from a relatively large distance without substantially altering existing genetic structure. This would allow managers to consider other factors (e.g., genetic diversity, environmental variation, flood tolerance, or flowering time) as potentially more important than genetic structure alone. Some research suggests that planting cuttings of *P. fremontii*, *S. gooddingii*, and *S. exigua* that come from the same location together increases overall performance of the newly established plants (Grady et al. 2017). If this is a priority and genetic structure differs among species, maintaining intact communities and basing plant material choices on genetic structure can be accomplished by using the genetic structure of the most differentiated species for decisions. In this case, this would mean collecting cuttings in tributaries to Kanab Creek (labeled as T6 for *S. exigua*, G7 for *P. fremontii*, and T1 for *S. gooddingii*), along the Paria River (labeled as M1 for *P. fremontii* and T1 for *S. exigua*), and along Tapeats Creek (T4 for *S. exigua*, G1 for *P. fremontii*), as these are locations where *P. fremontii* co-occurs with one or both of the *Salix* species.

Life History Traits and Genetic Differentiation

The results of this study imply that river connectivity may have a stronger influence than life history traits on genetic structure in riparian plants, but life history traits are nevertheless a valuable consideration, as previously demonstrated for many species (Hamrick & Godt 1996; Aguinagalde et al. 2005). *Salix exigua* exhibits substantial site-level genetic differentiation, despite no obvious regional genetic structure. While wind and water dispersal of seeds facilitates long-distance gene flow, insect pollination within patches of *S. exigua* may be increasing site-level differentiation. Long-distance seed dispersal and within-site pollination, then, separately facilitate and limit gene flow depending on the relative strength of their influence.

Prosopis glandulosa exhibits little admixture between genetic groups even though both groups occur within the same collection sites. It also exhibits significant site-level differentiation. This species is capable of selfing and has mixed populations of nectar-less and nectar-producing flowers (Lopez-Portillo et al. 1993; Bessega et al. 2000), which may explain these patterns. Alternatively, this genetic pattern may reflect differences between plants established pre- and post-dam, since dams can change genetic structure in riparian plants (Pollux et al. 2007; Werth et al. 2014).

For both of these insect-pollinated species, site-level differentiation could be leveraged to develop plant materials representing the full span of genetic variability in the region. Using materials from highly differentiated sites for restoration stock, identified with high pairwise F_{ST} values, would better mirror the genetic variability present in the region. Retaining this genetic variability provides the basis for evolutionary potential, which is an important consideration for the success of long-term restoration.

Genetic Diversity With Distance Downstream

Position along the river may be of little importance for genetic diversity in riparian plants. While there is theoretical support for multiple scenarios that result in higher diversity in downstream sections of river systems, these patterns seem to be stronger in aquatic species (Paz-Vinas et al. 2015). Rather than position along the river, population size may be a better tool for inferring genetic diversity in nonclonal species. While *S. gooddingii* does not generally conform to increasing genetic diversity with distance downstream, the three most downstream sites have the highest diversity. These sites also support large populations of *S. gooddingii*, which can also result in high diversity (Paz-Vinas et al. 2015).

If the management goal is to have high levels of genetic diversity, position along the river cannot be used to infer genetic diversity, rather site-level diversity estimates are needed. Targeting populations with known high genetic diversity and collecting from many sites would maximize genetic diversity. If plant materials are collected without knowledge of site genetic diversity, revegetation using vegetatively propagated, low genetic diversity stock can result in low genetic diversity riparian forests that remain on the landscape for many years (Smulders et al. 2008; Lin et al. 2009). Site-level assessments of genetic diversity are key to highlighting areas for conservation and potential source locations for plant propagation. For example, the low genetic diversity of the *P. fremontii* site G1 could result in not using this site for restoration stock elsewhere and/or to include cuttings from other sites if restoration occurs here. For *S. gooddingii*, the high genetic diversity sites T5, M2, and M3 could be targeted for cuttings to be planted throughout the study area in addition to local stock, given the low genetic structure in the region. For the other two species, this could mean ensuring that plant materials are developed from a variety of locations across the study location to increase restoration stock diversity.

Clonality and River Flows

Increased clonality is not associated with stabilized base flows from dam operations in *S. exigua*. Elsewhere stable flows increase clonality (e.g. Douhovnikoff et al. 2005; Smulders et al. 2008), suggesting that increased clonality varies by species or location. Arid and semiarid regions may tend to diverge from the general pattern, since obligate riparian species are generally

restricted to stable groundwater supplies (Butterfield et al. 2020), potentially resulting in similar cloning rates whether or not surface flows are variable. Alternatively, the dynamism of flash flood driven flows of tributaries in this region may increase clonality. Periodic, high-power floods that scour the river channel and deposit sediments could promote clonal growth (Mosner et al. 2012; Eusemann et al. 2013). The lack of consistency between clonality and river flows suggests that managers may need to assess the influence of river regulation on genetic patterns for each site individually, as extensive cloning affects genetic diversity and stand structure, and thus many restoration goals. Considering that climate change is associated with changes in low, median, and high river flows (Gudmundsson et al. 2021), links between clonality and river flow variability could benefit from further research.

The Role of Climate

While not directly tested in this study, the genetic patterns exhibited by *P. fremontii* are aligned with known differences in climate in this region. The sites sampled outside of the canyons are higher elevation, cooler, and genetically distinct from those inside the canyons. Genetic groups within the canyons follow a similar geographic pattern as the riparian floristic groups that grow along the Colorado River. The sites sampled in tributaries to Marble Canyon form a genetic group that is consistent with the cooler temperatures, mostly summer precipitation, and the riparian floristic group of Marble Canyon. The sites sampled in tributaries to Grand Canyon form a genetic group that is consistent with the warmer temperatures, bimodal precipitation pattern, and the floristic group of eastern Grand Canyon (Palmquist et al. 2018). The shift between these genetic groups occurs where the river bends around a high elevation uplift, changing precipitation patterns (Caster & Sankey 2016) which influence genetic patterns in *P. fremontii* (Cushman et al. 2014). Additionally, our sample sites occur at a possible junction of two genetically based ecotypes of *P. fremontii* (Ikeda et al. 2017) that are adapted to different temperatures (Grady et al. 2015; Cooper et al. 2019). The genetic patterns shown here could reflect the transition from one ecotype to the other.

Populations of broadly distributed plant species can have variable responses to environmental change leading to efforts to choose genotypes based on provenance climate variables (e.g. O'Neill et al. 2008; Grady et al. 2015). Broadly distributed plant species also tend to exhibit genetic structure across the landscape (e.g. Smulders et al. 2008; Cushman et al. 2014). By combining environmental differences with genetic patterns, restoration transfer zones can be refined to potentially augment restoration success (e.g. Durka et al. 2017; Massatti et al. 2020). This study indicates that for species crossing climatic gradients along large rivers, plants may be sourced from climatically different locations without altering existing genetic structure. This suggests that restoration practitioners have flexibility in choosing plant material based on the environment from which it comes without compromising evolutionary potential. For species growing along intermittent or small streams, trade-offs may need to be made between climate provenance and genetic structure, if genetically similar populations do not span climate gradients.

Research Needs for Restoration Guidelines

Guidelines for choosing riparian plant restoration material based on genetic structure and diversity would benefit from a review and meta-analysis of how consistently river connectivity, life history traits, and stabilized flow regimes correlate with genetic patterns. While this study is unique in characterizing the genetic patterns and life history traits of multiple species, inclusion of many more riparian species from across worldwide river systems would be required to disentangle interactions among the variables discussed here and historical and contemporary demographic processes. If enough riparian species with a broad array of life history traits, geographic distributions, and environmental conditions are characterized, it may be possible to elucidate general patterns of genetic structure in relation to characteristics unique to riparian areas.

Acknowledgments

Funding for this project was provided by Grand Canyon National Park (DUNS #806345542), the Glen Canyon Dam Adaptive Management Program administered by the U.S. Department of the Interior Bureau of Reclamation, and NSF Macrosystems Award #1340852. We thank many entities and people, including Grand Canyon National Park, Glen Canyon National Recreation Area, Navajo Nation, Hualapai Tribe, NAU Environmental Genetics and Genomics Laboratory, Grand Canyon Youth, NAU Cottonwood Group, Ogle Lab, M. Kearsley, J. Spence, L. Andrews, P. Rodrigues, N. Kent, E. Sukovich, T. Ojeda, B. Ralston, S. Sterner, A. Hazelton, L. Durning, A. Washuta, L. Gommerman, C. Fritzinger, S. Felder, C. Hackbarth, M. Sommer, E. Anderson, B. Wilson, R. Massatti, C. Roybal, N. Talkington, K. Smail, D. Hall, R. Seumptewa, M. Perkins, L. Fallon, M. O'Mara, J. Behan, C. Evans, D. Spice, J. Molton, C. McIntosh, J. Draper, and many other volunteers. Thanks to T. Gushue for basemaps. This manuscript is submitted for publication with the understanding that the U.S. government is authorized to reproduce and distribute reprints for governmental purposes. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. government. The authors have no conflicts of interest to declare.

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Supporting Information

The following information may be found in the online version of this article:

Supplement S1. Subregion details.
Supplement S2. Methods for estimating null alleles.
Supplement S3. Methods and results for NJ trees, *k*-means clustering, and DAPC.
Table S1. Collection site information, DNA voucher collections, and number of individuals sampled.
Table S2. List of loci used in statistical analyses for each species.
Table S3. The number of principle components and discriminant function retained in the discriminant analysis of principle components analysis.
Table S4. Analysis of molecular variance results, showing percent of variance explained (%) and *p*-values (*p*) for each species, based on genetic groups identified by STRUCTURE.
Table S5. Analysis of molecular variance results, showing percent of variance explained (%) and *p*-values (*p*) for each species, based on genetic groups identified by neighboring-joining (NJ) trees.
Table S6. Species-level genetic diversity and differentiation statistics.
Table S7. Overall genetic differentiation among sites measured by Weir and Cockerham FST, Nei's GST, G″ST, and D.
Table S8. *Populus fremontii* pairwise FST among sample sites.
Table S9. *Salix gooddingii* pairwise FST among sample sites.
Table S10. *Prosopis glandulosa* pairwise FST among sample sites.
Table S11. *Salix exigua* pairwise FST among sample sites.
Table S12. Diversity statistics for each species at the collection site level and genetic group level for species with well-supported neighbor-joining tree groups (>60 b.s.).
Table S13. Diversity estimates for each locus by species.
Figure S1. Output of Delta *K* analysis of STRUCTURE results for *Populus fremontii* using all collection sites. *K* = number of genetic groups.
Figure S2. Output of Delta *K* analysis of STRUCTURE results for *Populus fremontii* using only sites inside of Marble and Grand Canyons. *K* = number of genetic groups.
Figure S3. Output of Delta *K* analysis of STRUCTURE results for *Salix gooddingii*. *K* = number of genetic groups.
Figure S4. Output of Delta *K* analysis of STRUCTURE results for *Prosopis glandulosa*. *K* = number of genetic groups.
Figure S5. Output of Delta *K* analysis of STRUCTURE results for *Salix exigua*. *K* = number of genetic groups.
Figure S6. Neighbor-joining trees based on Hamming genetic distance and bootstrap values from 1000 trees.
Figure S7. Maps and bar plots illustrating genetic structure based on DAPC of neighbor-joining tree derived genetic groups.

Coordinating Editor: Catherine Febria

Received: 27 January, 2021; First decision: 17 March, 2021; Revised: 12 April, 2021; Accepted: 16 April, 2021