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Landscape genetic connectivity in a riparian foundation tree is jointly driven by climatic gradients and river networks

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Abstract. Fremont cottonwood (*Populus fremonti*) is a foundation riparian tree species that drives community structure and ecosystem processes in southwestern U.S. ecosystems. Despite its ecological importance, little is known about the ecological and environmental processes that shape its genetic diversity, structure, and landscape connectivity. Here, we combined molecular analyses of 82 populations including 1312 individual trees dispersed over the species' geographical distribution. We reduced the data set to 40 populations and 743 individuals to eliminate admixture with a sibling species, and used multivariate restricted optimization and reciprocal causal modeling to evaluate the effects of river network connectivity and climatic gradients on gene flow. Our results confirmed the following: First, gene flow of Fremont cottonwood is jointly controlled by the connectivity of the river network and gradients of seasonal precipitation. Second, gene flow is facilitated by mid-sized to large rivers, and is resisted by small streams and terrestrial uplands, with resistance to gene flow decreasing with river size. Third, genetic differentiation increases with cumulative differences in winter and spring precipitation. Our results suggest that ongoing fragmentation of riparian habitats will lead to a loss of landscape-level genetic connectivity, leading to increased inbreeding and the concomitant loss of genetic diversity in a foundation species. These genetic effects will cascade to a much larger community of organisms, some of which are threatened and endangered.

Key words: climate; conservation; Fremont cottonwood; gene flow; landscape genetics; landscape resistance; Populus fremontii; *reciprocal causal modeling*.

Introduction

Fremont cottonwood (*Populus fremontii*) is an important foundation tree species (Ellison et al. 2005) in riparian ecosystems of the U.S. Southwest, acting as a driver of community structure and ecosystem processes (Whitham et al. 2006). Common garden studies, for example, have shown that genotypic variation in Fremont cottonwood affects both community structure and diversity (Shuster et al. 2006, Ferrier et al. 2012, Bangert et al. 2013) and ecosystem processes such as aboveground net primary productivity (Grady et al. 2011). Studies of intraspecific genetic variation in other cottonwoods also demonstrate similar effects at both the community and ecosystem level (LeRoy et al. 2006, Bangert et al. 2008, Schweitzer et al. 2008, 2013, Keith et al. 2010, Busby et al. 2013). Despite their ecological importance, detailed studies of genetic diversity, structure, and landscape connectivity are lacking for any of the North American cottonwood species (Burczyk et al. 2004, Slavov and Zhelev 2010). Understanding how environmental and landscape-level features influence genetic variation and structure in foundation trees such as *P. fremontii* has important ecological and evolutionary consequences such as: (1) the identification of specific barriers to dispersal, or corridors that facilitate gene flow; (2) revealing how gene flow and genetic drift might promote or inhibit adaptive divergence among populations; and (3) elucidating how to best preserve genetic variation within broadly distributed tree species in order to maximize their adaptive potential for climate change (Grady et al. 2011).

Landscape genetics and population genomics provide methods to predict the effects of landscape structure and climatic gradients on genetic structure, population connectivity, and adaptive genetic variation (e.g., Cushman et al. 2006, 2013, Cushman and Landguth 2010, Shirk et al. 2010, Wasserman et al. 2010, 2012, Landguth and Cushman 2010, Landguth et al. 2011). Most past research in landscape genetics has focused on

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evaluating a few alternative models relative to null models such as isolation by distance or isolation by barriers (e.g., Coulon et al. 2004, Schwartz et al. 2009). As noted by Wasserman et al. (2010), observing that a resistance model has a higher correlation with genetic differentiation than does a null model of isolation by distance is a very weak basis to infer that it is the driver, and entails a large risk of errors of affirming the consequent (e.g., Cushman and Landguth 2010).

Several approaches have been used to improve model optimization in landscape genetics, including evaluating factorials of dozens to hundreds of alternative models and quantifying the unimodality of support (e.g., Cushman et al. 2006), conducting univariate optimization across scale and functional form (e.g., Wasserman et al. 2010), and conducting restricted multivariate optimization to seek stable predictions of optimal resistance parameters (e.g., Shirk et al. 2010). This latter approach is preferable for several reasons. By systematically varying each model parameter, the Shirk et al. (2010) approach enables researchers to identify a peak of support across a very large parameter space. Importantly, the approach accounts for interactions between variables, allows for nonlinear responses, and excludes variables that reduce model performance. In this paper we combined the multivariate restricted optimization approaches developed by Shirk et al. (2010) with the reciprocal causal modeling approach of Cushman et al. (2013) to evaluate the effects of river network connectivity and climatic gradients on genetic differentiation and gene flow in Fremont cottonwood across the U.S. Southwest. We evaluated the following two hypotheses: (1) Genetic differentiation is strongly related to the connectivity of the river network. Specifically, we expected that mid-sized to large rivers would facilitate gene flow, while small streams and terrestrial uplands will inhibit it. (2) Genetic differentiation is partly driven by climatic gradients. Because Fremont cottonwood occurs across a broad latitudinal gradientd we expected that gene flow would be attenuated between populations that experience different seasonal patterns of precipitation.

Testing these hypotheses is important because cottonwoods are dominants of an endangered ecosystem in the American Southwest (Noss et al. 1995), and knowledge of the factors that affect its population structure and connectivity is essential to guide effective conservation and restoration. To mitigate negative anthropogenic effects on riparian habitat, large and costly restoration projects are currently underway. For example, on 1030 km of the Lower Colorado River, a 50-year, $626 million riparian habitat restoration project was initiated in 2005 and managed by the Bureau of Reclamation (Follstad Shah et al. 2007, LCR MSCP 2010). Knowledge of how climate and river networks interact to affect genetic connectivity of a foundation tree species could play an important role in restoration strategies and in conserving the dependent communities they support.

Methods

Study species

Populus fremontii (Fremont cottonwood) is one of five North American *Populus* species and is broadly distributed along river corridors and tributaries throughout the southwestern United States (Eckenwalder 1977). It is an obligate outcrossing, dioecious (separate sexes), wind-pollinated tree species. Based on these life history traits, high connectivity among populations and substantial gene flow across its range is expected. In addition to wind pollination, seeds are also wind and water dispersed, which may further contribute to gene flow, although empirical data on seed dispersal are limited (Slavov et al. 2009).

Fremont cottonwood also hybridizes with other cottonwood species wherever species distributions overlap (Eckenwalder 1984), but extensive areas of non-overlapping Fremont populations still occur. Along the western front of the Colorado Rocky Mountains, USA, Fremont cottonwood partially overlaps with another closely related and morphologically similar species, *P. deltoides* (eastern cottonwood). Although morphological characters allow taxonomic discrimination of the two species (Eckenwalder 1977), there is considerable overlap among these characters, suggesting that the two species are either a single, morphologically variable taxon, or that hybridization has played a role in their evolutionary history. To constrain our study to Fremont cottonwood, we sampled throughout its range, but removed populations that showed genetic admixture between the two species (see *Genetic admixture* below).

Sampling of cottonwood populations

Leaf samples from 71 populations were collected throughout the southwestern U.S. range of Fremont cottonwood (Fig. 1; Appendix A: Table A1). Samples were dried using Drierite (Drierite, Xenia, Ohio, USA) and stored at room temperature. Geographic coordinates were recorded using a GPS unit for most samples. Where satellite signal was unavailable, locality data were determined using topographic maps. For comparison, included in this sample set were 11 populations ($N = 66$) of eastern cottonwood from a 2350-km transect extending from Tucumcari, New Mexico, to Columbus, Ohio, USA. Together with the 71 Fremont populations, a total of 82 populations encompassing 1312 individuals was used to assess the degree of genetic differentiation between *P. fremontii* and *P. deltoides* and to quantify the degree to which hybridization, assessed as genetic admixture, occurs.

DNA extraction and simple sequence repeat analysis

For each sample, ~6 g of dried leaf material was dry-milled using 2-mm Sintered Zirconium silicate grinding media (GlenMills, Clifton, New Jersey, USA) and

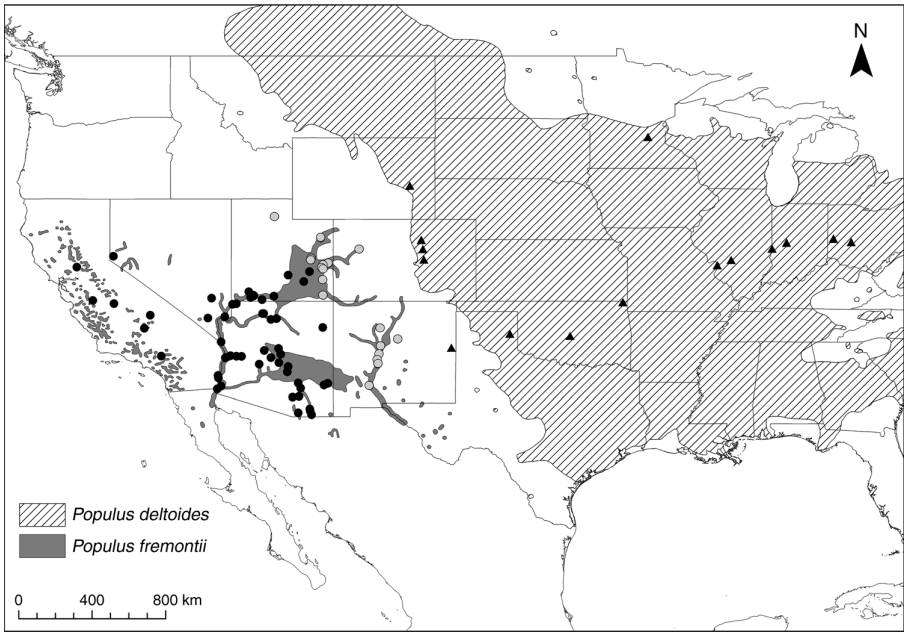

FIG. 1. Ranges of Fremont cottonwood (*Populus fremontii*) and eastern cottonwood (*Populus deltoides*) populations sampled (Little 1971). *P. deltoides* samples are shown in black triangles. *P. fremontii* used in the landscape genetic analysis are shown in black circles. Populations showing admixture between *P. deltoides* and *P. fremontii* are shown in gray circles. These were excluded from the landscape genetic analysis.

shaken vigorously using the 2000 Geno/Grinder (SPEX, SamplePrep, Metuchen, New Jersey, USA). The pulverized material was then used in whole genomic DNA extractions following the DNeasy 96 Plant Mini Kit protocol (Qiagen, Valencia, California, USA). DNA was quantified using NanoDrop ND-1000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA).

Based on an initial screening of 25 simple sequence repeat (SSR) loci, 13 SSRs were chosen from the Populus SSR Resource database (Appendix A: Table A1; Tuskan et al. 2004; International *Populus* Genome Consortium, *available online*).[8] Touchdown polymerase chain reaction (PCR) amplification was performed in 10 μL volumes, with 12.5 ng template DNA, 0.15 mM dNTPs deoxynucleotide triphosphates), 0.35 units Taq polymerase, 1× PCR buffer, and 2.5 mM $MgCl_2$. Thermal cycling conditions were: 94°C for 5 min (one cycle); 95°C for 15 s, 60°C for 15 s (decrease 1°C each cycle), 72°C for 30 s (10 cycles); 95°C for 15 s, 50°C min for 15 s, 72°C for 30 s (25 cycles); with a final cycle of 72°C for 10 min. Forward primers were end-labeled with either FAM (fluorescein amidite), NED, PET, or VIC fluorescent dye (Applied Biosystems [AB], Foster City, California, USA). An AB 3730xl Genetic Analyzer was used for fragment analysis of PCR products with an internal size standard (GeneScan LIZ600; Foster City, California, USA). Allele fragment sizes were scored using AB GeneMapper v4.0 (Applied Biosystems 2011) and automatic scoring using assigned bins. All alleles were manually checked for accuracy.

[8] http://www.ornl.gov/sci/ipgc/ssr_resource.htm

Regional assessment of genetic structure and admixture

The program STRUCTURE v.2.3.3 (Pritchard et al. 2000, Falush et al. 2003) was used to infer population structure and assess genetic admixture without a priori assignment of the number of populations. A burn-in of 15 000 and values of $K = 1$–20 were tested. The best fit K value was estimated using the ΔK statistic (Evanno et al. 2005), implemented in STRUCTURE HARVESTER (Earl and vonHoltd 2011). The program CLUMP (Jakobsson and Rosenberg 2007) was used to combine the results of the replicate runs, and DISTRUCT (Rosenberg 2004) was used to create visual images.

Genetic admixture

Preliminary assessment of genetic diversity and structure among the 82 populations revealed that some populations in eastern Utah, one in eastern Arizona, and all populations in Colorado and New Mexico were more genetically similar to eastern cottonwood. A few populations in eastern Utah showed genetic admixture, suggesting that hybridization between the two species occurs. The percentage of admixture in these hybrid populations ranged from 1% to 99.1%. We removed populations that showed greater than 10% admixture with eastern cottonwood, resulting in landscape genetic

analysis of 40 Fremont cottonwood populations encompassing 743 individual trees.

Genetic diversity and differentiation in Fremont cottonwood

Among population differentiation (F_{ST}; Wright 1965) was calculated and an analysis of molecular variance (AMOVA) was conducted using GenAlEx v6.1 (Peakall and Smouse 2006). Pairwise F_{ST} was also estimated for all populations, and a Mantel Test (Mantel 1967) was used to test whether pairwise F_{ST} was correlated with geographic distance across all populations and loci. Geographic distance matrix calculations were made using a central point designated for each Fremont cottonwood population and determined by averaging the GPS coordinates of the entire population (Fig. 1).

Reciprocal causal modeling

The predominant analytical approach to associate landscape patterns with gene flow processes is based on pairwise calculation of cost distances, using least cost paths (e.g., Coulon et al. 2004, Cushman et al. 2006) or multi-path circuit approaches (McRae 2006) followed by application of Mantel and partial Mantel tests (Mantel 1967, Smouse et al. 1986) to correlate pairwise genetic distances with pairwise cost distances for alternative resistance models. There has been controversy in the literature about the appropriateness of Mantel testing in landscape genetics (e.g., Raufaste and Rousset 2001, Castellano and Balletto 2002). Legendre and Fortin (2010) clarified this confusion, and argue that, while distance-based regression approaches, such as the Mantel test, have lower power than traditional linear models, they remain the appropriate framework when the hypotheses are explicitly defined in terms of distance matrices, as they are in landscape genetic analyses testing effects of landscape resistance on neutral genetic differentiation. Recently, Guillot and Rousset (2011) reported that partial Mantel tests may be biased when there is spatial correlation in landscape resistance. Autocorrelation deriving from isolation by distance (Meirmans 2012) and isolation by resistance (Amos et al. 2012) leads to elevated Type I error rates in Mantel tests.

The causal modeling framework has been widely used as a model selection and hypothesis testing procedure in landscape genetics (Cushman et al. 2006). The Cushman et al. (2006) approach involves identifying the most supported resistance hypothesis among a range of alternative resistance models (based on statistical significance or magnitude of the Mantel r), and then using partial Mantel tests (Legendre and Troussellier 1988, Legendre 1993) to determine whether it meets the statistical expectations of a causal model relative to alternative models of isolation by distance or isolation by barrier. Cushman and Landguth (2010) evaluated the power of this framework and found it to perform well in identifying the drivers of genetic differentiation in a complex landscape, and rejecting incorrect and correlated alternatives. Cushman et al. (2013) further evaluated the reliability of the causal modeling approach using partial Mantel tests in landscape genetics and found that causal modeling improves but does not eliminate elevated Type I error rates. They proposed an alternative approach, called reciprocal causal modeling, which greatly improves the ability to correctly identify the drivers of genetic differentiation and reject highly correlated alternative hypotheses.

In each phase of the analysis, we used reciprocal causal modeling (Cushman et al. 2013) to compete all hypotheses at that step with each other, and identify the hypothesis in the set that was uniquely supported relative to the others. The reciprocal causal modeling approach works by computing all combinations of partial Mantel tests in the set of alternative hypotheses (each hypothesis partialling out each other hypothesis). Then for each combination of hypotheses, we computed the difference in the magnitude of the partial Mantel r between hypothesis A partialling out hypothesis B, and hypothesis B partialling out hypothesis A. If hypothesis A is correct, then $(A \mid B - B \mid A)$ should be positive. Conversely, if hypothesis B is correct, then $(A \mid B - B \mid A)$ should be negative. We computed a matrix of these differences in the magnitude of partial Mantel r, with the focal hypotheses along the x-axis and the alternative hypotheses along the y-axis. A model that is fully supported in reciprocal causal modeling would have all positive values along the y-axis (it is supported independently of all other models) and all negative values along the x-axis (no alternative models are supported independently of it). At each step of the analyses, we computed these reciprocal causal modeling matrices, identified the uniquely supported candidate model, and passed that model on as the starting point for the next step. In this way, we combined reciprocal causal modeling to evaluate models (Cushman et al. 2013), with iterative model optimization (Shirk et al. 2010) to maximize the fit of the resistance model to observed genetic differentiation.

Organizational models.—Our analysis involved optimizing the relationship between landscape features (rivers, uplands, and climate gradients) and gene flow in a series of nested steps. We combined restricted optimization (Shirk et al. 2010) with reciprocal causal modeling (Cushman et al. 2013) to evaluate a large number of alternative resistance models. Testing our first hypothesis involved optimizing the relative resistance of streams and rivers relative to terrestrial uplands (Fig. 2). There are four steps in our test of hypothesis 1. First, we used reciprocal causal modeling on nine hypotheses of the relationship between river order and cottonwood gene flow (Fig. 2a). In the second step, we took the resistance model most supported in step 1 and used reciprocal causal modeling to evaluate 15 variations in which the relative resistance of the orders of river size found to be influential in step 1 (Fig. 2b). The

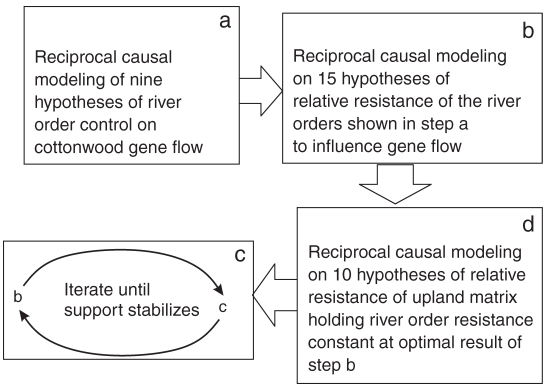

FIG. 2. First phase of the analysis involved optimizing the relative resistance of streams and rivers relative to terrestrial uplands. (a) We used reciprocal causal modeling to evaluate nine hypotheses of river order control on gene flow. Based on the results of that step, (b) we used reciprocal causal modeling to evaluate 15 additional hypotheses of the relative resistance of river orders. Next, (c) we evaluated 10 hypotheses of relative resistance of terrestrial uplands. Finally, we iterated steps (panels b and c) until convergence to a stable solution.

third step used reciprocal causal modeling on 10 hypotheses that held the relative resistance of different river orders constant at the optimal combination identified in step 2, but varied the resistance of the terrestrial upland matrix. The fourth step was an iterative repeat of steps b and c until convergence to a stable solution. This combination of iterative restricted optimization (similar to that proposed by Shirk et al. 2010) and reciprocal causal modeling (Cushman et al. 2013) provides a strong means to evaluate relative support for alternative hypotheses and efficiently optimize resistance estimates for gene flow as functions of river order and network connectivity.

Testing our second hypothesis involved optimizing the relationships between climate gradients and genetic differentiation of Fremont cottonwood (Fig. 3). There were three steps in our test of hypothesis 2. The first step was broken into five sub-steps (step 1a, step 1b, step 1c, step 1d, step 1e; Fig. 3). The first sub-step (step 1a; Fig. 3) used reciprocal causal modeling on seven hypotheses of control of gene flow by climate cluster membership (Table 1). The second sub-step (step 1b; Fig. 3) used reciprocal causal modeling on nine hypotheses of control of gene flow by pairwise differences in seasonal precipitation. The third, fourth, and fifth sub-steps (step 1c, step 1d, step 1e, respectively; Fig. 3) used reciprocal causal modeling to test 21 hypotheses (in each sub-step) of control of gene flow by cumulative path differences in monsoon, spring, and winter precipitation, respectively. The second step of the climate–gene flow analysis used reciprocal causal modeling to optimize the relative influence of cumulative path differences in monsoon, spring, and winter precipitation (step 2; Fig. 3). The third step of the climate–gene-flow analysis used reciprocal causal modeling to combine supported models from step 1a, step 1b, and step 2 into a final model of effects of climate on gene flow.

The final phase of the analysis sought to optimize the relative influence of river order and upland resistance compared to resistance to gene flow presented by climatic differences. In this third phase we used reciprocal causal modeling to test 202 alternative hypotheses of the relative effects of climate vs. river network connectivity on gene flow (Fig. 4). The 202 hypotheses varied the relative weight of the optimized climate resistance model across multiples from 1 to 200 times larger relative influence than the optimized river order resistance model. The two additional hypotheses (making 202) evaluated in this phase are isolation by distance and isolation by Bayesian cluster membership.

Developing river order resistance hypotheses.—Given that Fremont cottonwood is primarily wind dispersed and that seeds require streams and rivers for recruitment, gene flow is likely to follow major river and stream tributary corridors. Thus, stream order was chosen in this study as a major landscape feature acting as a conduit for gene flow. Strahler stream order (Strahler 1957) was obtained through the National Hydrology Dataset plus data (NHD plus; *available online*).[9] Strahler stream order classifies streams in a hierarchical manner by size. Quantification of Strahler stream order class sizes were calculated using an ArcGIS 10 hydrology algorithm (Gleyzer et al. 2004). Layers corresponding to sample collections were downloaded from NHD plus and stream layers were created in ArcGis 10 (ESRI 2011). The stream order classification is map scale dependent; the order for each stream in the system is related to the resolution of the map and number of drainages included. The data were then joined in ArcGIS 10 to generate one large data set to ensure resolution was the same for all of the stream segments. The maximum stream order within this study area was stream order seven. Finally, after applying our hypothesized resistance factors based on stream order, we used the stream order network to calculate the least cost path between all pairs of sample locations, and produced a cost distance matrix for each hypothesis tested.

Developing climate resistance hypotheses.—We used GridCalc (*available online*)[10] to aggregate PRISM data (*available online*)[11] into a monthly 30-year window from which precipitation averages and temperature extremes were extracted. These extracted values were compiled into spatially explicit American Standard Code for Information Interchange (ASCII) images for each month and climate variable. Further processing with ArcGIS' raster calculator enabled these individual images to be grouped into five seasonalities: winter, spring, summer, monsoon, and fall.

[9] http://nhd.usgs.gov/
[10] www4.nau.edu/direnet/methods/
[11] http://www.prism.oregonstate.edu/

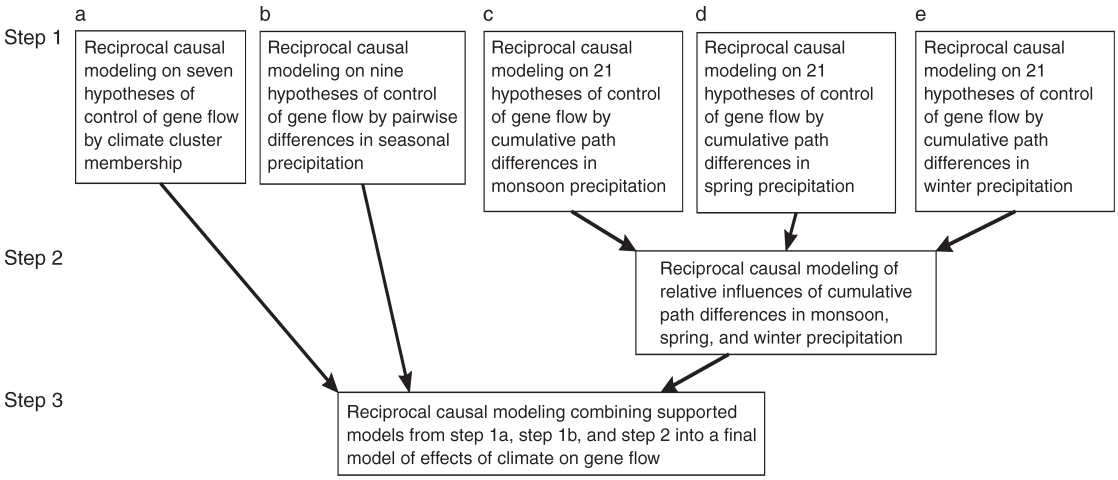

FIG. 3. Optimizing the relationships between climate gradients and genetic differentiation of Fremont cottonwood. The first step was broken into five sub-steps (step 1a, step 1b, step 1c, step 1d, and step 1e): (a) reciprocal causal modeling on seven hypotheses of control of gene flow by climate cluster membership, (b) reciprocal causal modeling on nine hypotheses of control of gene flow by pairwise differences in seasonal precipitation, (c, d, e) reciprocal causal modeling to test 21 hypotheses (in each substep) of control of gene flow by cumulative path differences in monsoon, spring, and winter precipitation, respectively. The second step (step 2) of the climate–gene flow analysis used reciprocal causal modeling to optimize the relative influence of cumulative path differences in monsoon, spring, and winter precipitation. The third step (step 3) of the climate–gene flow analysis used reciprocal causal modeling to combine supported models from step 1a, step 1b, and step 2 into a final model of effects of climate on gene flow.

We evaluated three sets of climate-based resistance hypotheses. The first set of these proposed that genetic differentiation of Fremont cottonwood populations would be associated with regional zones of similar climate, such that populations within spatial clusters of similar climate would show low levels of genetic differentiation relative to one another, while populations residing in different climate zones would have divergent structure. This would reflect local adaptation to local climate. We constructed these hypotheses by computing Isocluster (ESRI 2012) clusters of areas of similar climate based on winter, spring, and monsoon precipitation. We evaluated five levels of climate clustering and its association with Fremont cottonwood gene flow. In each of these, we produced a model matrix, which reported whether or not each pair of sampled cottonwood populations were in the same or different climate clusters. These matrices were then used as the independent variable in reciprocal causal modeling analyses, as described in the *Organizational models* section above.

The second set of climate hypotheses proposed that genetic differentiation is a continuous function of pairwise differences in seasonal precipitation, such that populations that have similar seasonal precipitation profiles will have similar genetic structure. This would reflect continuous variation in local adaptation to precipitation. We evaluated this by computing the pairwise differences in winter, spring, monsoon, and annual precipitation for each combination of sampled populations, and using this matrix as the independent

TABLE 1. Resistance parameters for the step 1 reciprocal causal modeling of the effects of river network on gene flow.

	Resistance hypotheses							
River order	dist	1r20	2r20	3r20	4r20	5r20	6r20	7r20
Terrestrial uplands	1	20	20	20	20	20	20	
1	1	1	20	20	20	20	20	20
2	1	1	1	20	20	20	20	20
3	1	1	1	1	20	20	20	20
4	1	1	1	1	1	20	20	20
5	1	1	1	1	1	1	20	20
6	1	1	1	1	1	1	1	20
7	1	1	1	1	1	1	1	1

Notes: We tested nine hypotheses in step 1: struct, isolation by structure groups; dist, isolation by distance; 1r20–7r20, isolation by landscape resistance where river network provides low resistance and terrestrial uplands are high resistance, with river network defined as a gradient from all streams (orders 1–7; e.g., 1r20) to only the largest streams (order 7; e.g., 7r20). The values in the cells represent the relative resistance of rivers of different orders for each hypothesis. The isolation by structure groupings is not shown, as that hypothesis does not assign resistance based on landscape conditions, but based on structure group membership.

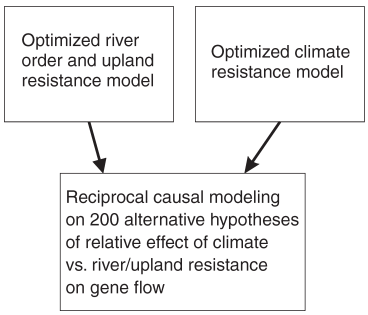

FIG. 4. Optimizing the relative influence of river order and upland resistance compared to resistance to gene flow presented by climatic differences.

variable in reciprocal causal modeling as described in the *Organizational models* section above.

The third set of climate hypotheses proposes that genetic differentiation is a continuous function of cumulative isolation by climatic differences between pairs of populations. This would result in continuous genetic differentiation as functions of cumulative difference in seasonal precipitation across the landscapes separating pairs of sampled populations. We evaluated this hypothesis by calculating the local slope (in a 5×5 window) of spatial change in seasonal precipitation (winter, spring, monsoon) and calculating the cumulative cost distance across these precipitation slope surfaces (e.g., Yang et al. 2013), and used these cost distance matrices as independent variables in the reciprocal causal modeling as described in the *Organizational models* section above.

RESULTS

Regional patterns of genetic structure and admixture

Although low regional structure in *P. fremontii* was expected due to wind-dispersed life history traits across its widespread distribution, STRUCTURE analysis showed distinct regional groupings. The best statistical fit was a significant value of ΔK at $K = 2$, and a secondary peak at $K = 4$. A neighbor-joining distance analysis also showed regional groupings (data not shown). Two distinct groupings occurred, delineating Fremont from eastern cottonwood. Some populations showed admixture between the two species, while other populations grouped entirely with Fremont cottonwood. The admixed and eastern cottonwood populations included populations along the eastern edge of the Fremont cottonwood range, including some in eastern Utah, and all Colorado and New Mexico populations. These two major groupings support the STRUCTURE analysis plot of ΔK at $K = 2$ above.

After removing all eastern cottonwood populations including those exhibiting admixture greater than 0.1, a ΔK at $K = 3$ was the only significant STRUCTURE grouping for remaining populations of Fremont cottonwood. In STRUCTURE, with the removal of admixed populations greater than 10%, Fremont cottonwood cluster groupings remained the same.

The AMOVA analysis showed 75% of the genetic diversity was distributed within individuals, 3% among individuals, and 22% among populations. There was significant genetic differentiation among subpopulations relative to total population variation ($F_{ST} = 0.221$, $P < 0.001$). Regional groupings corresponding to results based on STRUCTURE analysis were also found to be highly significant ($\phi_{RT} = 0.26$, $P < 0.001$ [where ϕ_{RT} is the among population component of genetic variation within the region studied]).

Hypothesis 1: river network resistance optimization.— The first step in the optimization of river network resistance involved reciprocal causal modeling on nine alternative hypotheses, which included seven variations in which orders of stream/river size were predicted to facilitate gene flow of Fremont cottonwood (Table 1), as well as isolation by distance and isolation by STRUCTURE Bayesian clustering. The results of the first step of river order resistance optimization with reciprocal causal modeling indicated that streams of order 2 and larger have a positive effect on gene flow, while first-order streams do not (Appendix B: Fig. B1). It also indicated that isolation by distance and isolation by STRUCTURE groupings were not supported independently of any river order resistance hypotheses.

The second step of river network optimization evaluated the relative resistance of streams of order 2–7 across 15 alternative hypotheses (Table 2). Reciprocal causal modeling identified the 13th of these alternative hypotheses as independently supported (Appendix B: Fig. B2). In this hypothesis, rivers of order 5, 6, and 7 equally facilitate gene flow, with fourth-order streams 3.8 times more resistant, third-order streams 7.5 times more resistant, and second-order streams 11.3 times more resistant than these larger rivers. All other areas (first-order streams and terrestrial uplands) were assigned a resistance 15 times greater than these lowest resistance larger rivers.

The third step of river network optimization evaluated 14 alternative models of relative resistance of first order streams and terrestrial uplands compared to rivers of orders 2–7, holding these at the optimal relative resistance identified in step 2, and varying the relative resistance of first order streams and uplands from 12 to 25 times higher than the lowest resistance large rivers (Table 3; Appendix B: Fig. B3). Reciprocal causal modeling identified the first of these hypotheses (13a) as independently supported (Appendix B: Fig. B3). This hypothesis suggests that first-order streams and terrestrial uplands are 12 times more resistant than fifth-, sixth-, or seventh-order rivers.

The fourth step re-evaluated the step 2 hypotheses of relative river order resistance, but with the resistance of first-order streams and terrestrial uplands set to 12, as identified in step 3 (Table 4). This model suggests that terrestrial uplands are 12 times more resistant than the

TABLE 2. Resistance parameters for the step 2 reciprocal causal modeling of the effects of river network on gene flow.

River order	Resistance model														
	r1	r2	r3	r4	r5	r6	r7	r8	r9	r10	r11	r12	r13	r14	r15
7	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	17	10	10	10	10	12	10	10	10	10	25
5	10	10	10	20	33	10	10	10	10	17	10	10	10	30	50
4	10	10	25	40	50	10	10	13	20	25	10	10	38	60	75
3	10	33	50	60	67	10	17	25	30	33	10	50	75	90	100
2	10	67	75	80	83	25	33	38	40	42	75	100	113	120	125
Other	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150

Notes: Step 1 identified river order 2–7 as facilitating gene flow and terrestrial uplands, and streams of order 1 providing high resistance. In step 2, we tested 15 additional hypotheses to evaluate the relative resistance of each stream order to gene flow: r1–r15 represent 15 alternative hypotheses for the relative effect of different stream orders on landscape resistance. Model r1 proposes resistance of river orders 2–7 are the same, and are 15 times less than terrestrial uplands. Conversely, r15 suggests that the resistance of river order 6 is 2.5 times higher, river order 5 is 5 times higher, river order 4 is 7.5 times higher, river order 3 is 10 times higher, river order 2 is 12.5 times higher, and terrestrial uplands is 15 times higher than resistance of rivers of order 7.

largest rivers (orders 5, 6, 7), with resistance of rivers of different order as in model 13 from the second step of the causal modeling. This confirms convergence to a stable solution of relative resistance of river orders and terrestrial uplands, and ends the optimization loop for resistance of the river network relative to terrestrial uplands (Appendix B: Fig. B4).

Hypothesis 2: climate gradient optimization.—We evaluated three sets of alternative hypotheses about genetic differentiation as functions of seasonal precipitation. In the first set, we competed seven hypotheses about climate clusters (five clusters) driving genetic differentiation relative to each other and isolation by distance and isolation by STRUCTURE groups (Appendix B: Fig. B5). None of the climate cluster hypotheses were supported independently of isolation by STRUCTURE groups or isolation by distance, and both isolation by STRUCTURE group and isolation by distance were supported independently of all climate cluster hypotheses.

In the second set of hypotheses of genetic differentiation as functions of climate gradients, we evaluated nine hypotheses of pairwise differences in seasonal precipitation (seven combinations) relative to each other and isolation by distance and isolation by STRUCTURE groups (Appendix B: Fig. B6). None of the seasonal differences in precipitation hypotheses were supported independently of isolation by STRUCTURE groups or isolation by distance, and both isolation by STRUCTURE group and isolation by distance were supported independently of all precipitation difference hypotheses.

In the third set of hypotheses of genetic differentiation as functions of climate gradients, we evaluated hypotheses of genetic isolation by gradients of cumulative difference in seasonal precipitation between pairs of sampled populations. We evaluated each season separately in the first step. In this first step we evaluated 19 different forms of resistance as functions of the slope of each season's precipitation. These functional forms were power functions from 0.2 to 2.0 power at steps of 0.1, reflecting different "shapes" of resistance as a function of slope of change in precipitation across the landscape (e.g., Shirk et al. 2010, Wasserman et al. 2010). None of the power functions of slope of change in monsoon precipitation across the study area were supported independently of isolation by STRUCTURE groups, and both isolation by distance and isolation by STRUCTURE group are supported independently of all slope of monsoon precipitation hypotheses (Appendix B: Fig. B7). Among models of genetic differentiation as functions of slope of spring precipitation, we found that model s03 was supported independently of all other slope of spring precipitation hypotheses and indepen-

TABLE 3. Resistance parameters for the step 3 reciprocal causal modeling of the effects of river network on gene flow.

River order	Resistance model													
	13a	13b	13c	13d	13e	13f	13g	13h	13i	13j	13k	13l	13m	13n
7	10	10	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4	38	38	38	38	38	38	38	38	38	38	38	38	38	38
3	75	75	75	75	75	75	75	75	75	75	75	75	75	75
2	113	113	113	113	113	113	113	113	113	113	113	113	113	113
Other	120	130	140	150	160	170	180	190	200	210	220	230	240	250

Notes: Step 2 identified model 13 as the most supported model of the relative resistance of different river order. In step 3, we evaluated 15 alternative models for the relative resistance of the terrestrial uplands relative to river network. Model 13a suggests that terrestrial uplands are 12 times more resistant than rivers of orders 5, 6, and 7. Conversely, 13n suggests that terrestrial uplands are 25 time more resistant than the least resistant rivers.

TABLE 4. Resistance parameters for the step 4 reciprocal causal modeling of the effects of river network on gene flow.

River order	Resistance model														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
7	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	17	10	10	10	10	12	10	10	10	10	25
5	10	10	10	20	33	10	10	10	10	17	10	10	10	30	50
4	10	10	25	40	50	10	10	13	20	25	10	10	38	60	75
3	10	33	50	60	67	10	17	25	30	33	10	50	75	90	100
2	10	67	75	80	83	25	33	38	40	42	75	100	113	120	125
Other	120	120	120	120	120	120	120	120	120	120	120	120	120	120	120

Notes: Step 3 indicated that terrestrial uplands are 12 times more resistant than the least resistant rivers and that river orders were relatively resistant as indicated in step 2 model 13. To confirm this solution is a stable convergence, we evaluated the step 2 hypotheses of relative river order resistance combined with the step 3 relative resistance of terrestrial uplands.

dently of isolation by distance and isolation by STRUCTURE groups (Appendix B: Fig. B8). No other models were supported independently of s03. This model suggests that genetic differentiation is correlated with cumulative cost of moving across a grid of (slope spring precipitation)$^{0.3}$. Among models of genetic differentiation as functions of slope of winter precipitation, we found that models w04 and w05 were both approximately equally supported independently of all other slope of spring precipitation hypotheses, except each other, and independently of isolation by distance and isolation by STRUCTURE groups (Appendix B: Fig. B9). No other models were supported independently of w04 or w05. Given that w05 was more strongly supported relative to isolation by distance, we chose this model as most supported in this reciprocal causal modeling analysis. This model suggests that genetic differentiation is correlated with cumulative cost of moving across a grid of (slope winter precipitation)$^{0.5}$.

In the second step of the optimization of genetic differentiation as functions of slope of seasonal precipitation, we combined the resistance models supported for spring and winter in 11 combinations of relative influence (Appendix B: Fig. B10). These combinations varied the influence of slope of change of spring vs. winter precipitation (as optimized in the first step of the analysis). Model s1w5 was supported independently of all other combined slope of seasonal precipitation hypotheses, and no other models are supported independently of S1W5. This model suggests that spatial variation in winter precipitation has five times greater influence on gene flow than variation in spring precipitation. This step ended the optimization of resistance to gene flow as functions of climate gradients.

The final step in the optimization of the resistance model for Fremont cottonwood gene flow sought to evaluate the relative weight of the optimized river network resistance model compared to the optimized climate gradient resistance model. We evaluated 200 combinations of relative weight, plus isolation by distance and isolation by STRUCTURE group (Fig. 5). Model R1C74 was supported independently of all other combined river resistance and seasonal precipitation hypotheses, and no other models were supported independently of R1C74. This model was also supported independently of isolation by distance and isolation by STRUCTURE group. This model suggests that a combined hypothesis of spatial variation in seasonal precipitation (hypothesis s1w5) has 74 times greater weight of influence on gene flow than resistance of the river network (hypothesis 13a). It is important to note that this does not indicate that climate is 74 times more important than river network, as the scales of the variables are different (e.g., the slope model was raised to the 0.3 or 0.5 power, resulting in small resistance values, while the river order network model ranged on a scale from 10 to 120). This final reciprocal causal modeling analysis, however, provides the optimal weights to combine the river order and climate hypotheses into a single resistance layer. We did this by multiplying the climate resistance layer by 74, adding it to the optimized river network resistance model, and rescaling by dividing by the minimum of this combined layer to produce a final resistance layer with a minimum of 1 (Fig. 6).

DISCUSSION

River order and gene flow

Consistent with our first hypothesis, we found that gene flow is facilitated by mid-sized to large rivers, and is resisted by small streams and terrestrial uplands. Specifically, streams and rivers of second order and larger provide lower resistance to gene flow than the surrounding terrestrial uplands. We expected this based on the life history of Fremont cottonwood, which forms continuous woodlands in the floodplains of mid-sized to large rivers, but is intermittent along small streams, and is generally absent in terrestrial uplands (Eckenwalder 1977).

We found that resistance to gene flow decreases with increasing river size. Specifically, rivers of order 5, 6, and 7 (the largest in the study area) all were found to have equally low resistance to gene flow, while streams of order 4 were nearly four times, order 3 were 7.5 times, and order 2 over 11 times as resistant as the largest rivers. This shows a strong, nonlinear change in resistance to gene flow with river size, such that medium

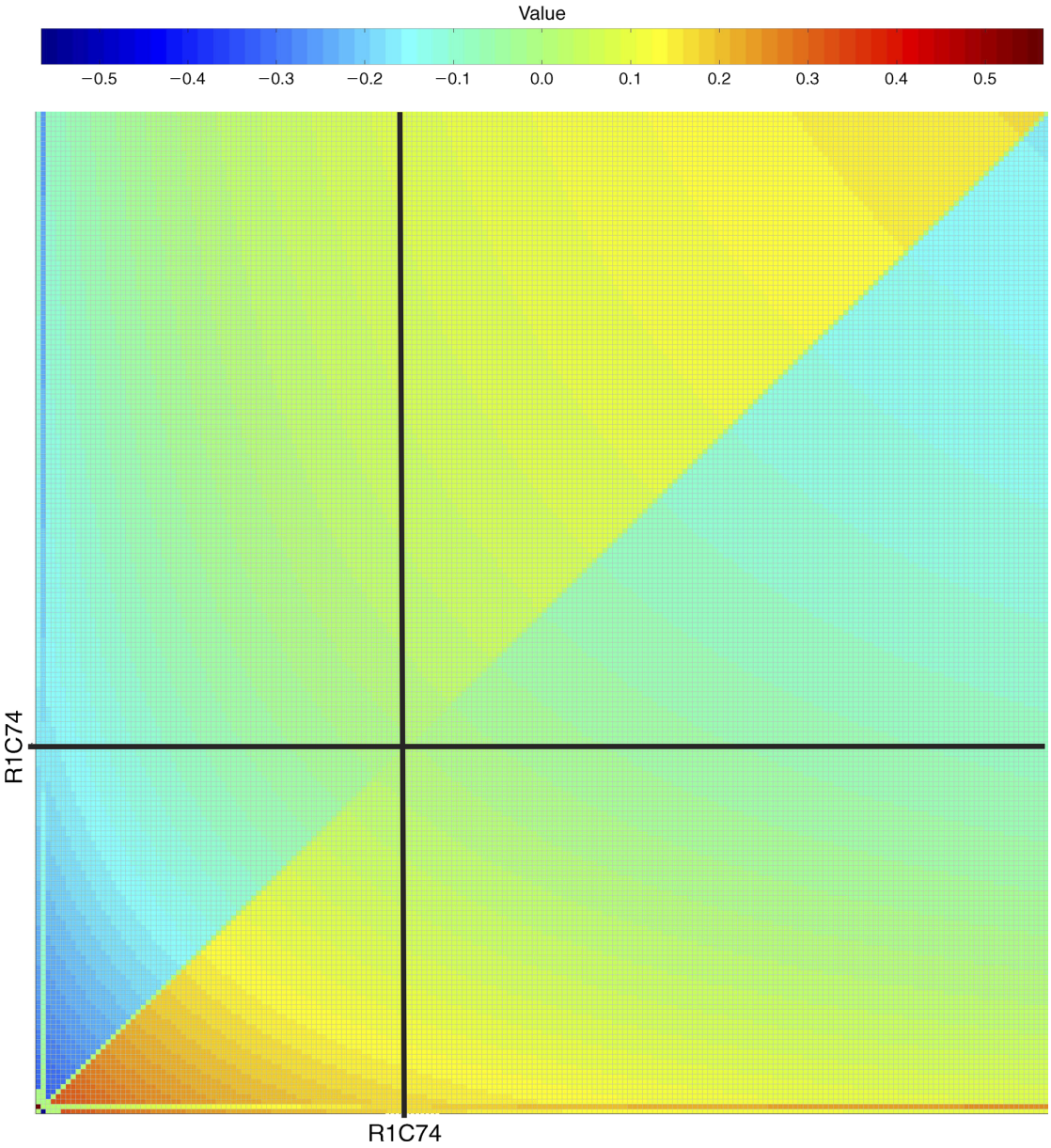

FIG. 5. Reciprocal causal modeling to test 202 hypotheses about the relative influence of river network connectivity and spatial variation in winter–spring precipitation on gene flow. The 202 hypotheses include isolation by distance and isolation by structure groups, and also vary the relative effect of precipitation compared to river network connectivity. The relative resistance of the river network is as specified in hypothesis 13 (Table 4; Appendix B: Fig. B4). The relative resistance of spatial variation in seasonal precipitation is as specified in hypothesis S1W5 (Appendix B: Fig. B10). The reciprocal causal modeling shown here varies the relative weight of S1W5 relative to river resistance hypothesis 13 across 200 levels or relative effect corresponding to 1, 2, 3 . . . 200 times more weight to S1W5 than river resistance hypothesis 13. Cell values indicate reciprocal causal modeling score (x model $|$ y model $-$ y model $|$ x model). A fully supported hypothesis would have all positive reciprocal causal modeling scores in the vertical dimension (e.g., the model is supported independently of all others) and all negative scores in the horizontal dimension (e.g., no other models are supported independently of the model). Model R1C74 is fully supported based on these criteria, and is supported independently of all other combined river resistance and seasonal precipitation hypotheses, and no other models are supported independently of R1C74. This model suggests that a combined hypothesis where spatial variation in seasonal precipitation (hypothesis S1W5) has 74 times greater weight than influence on gene flow than resistance of the river network (hypothesis 13).

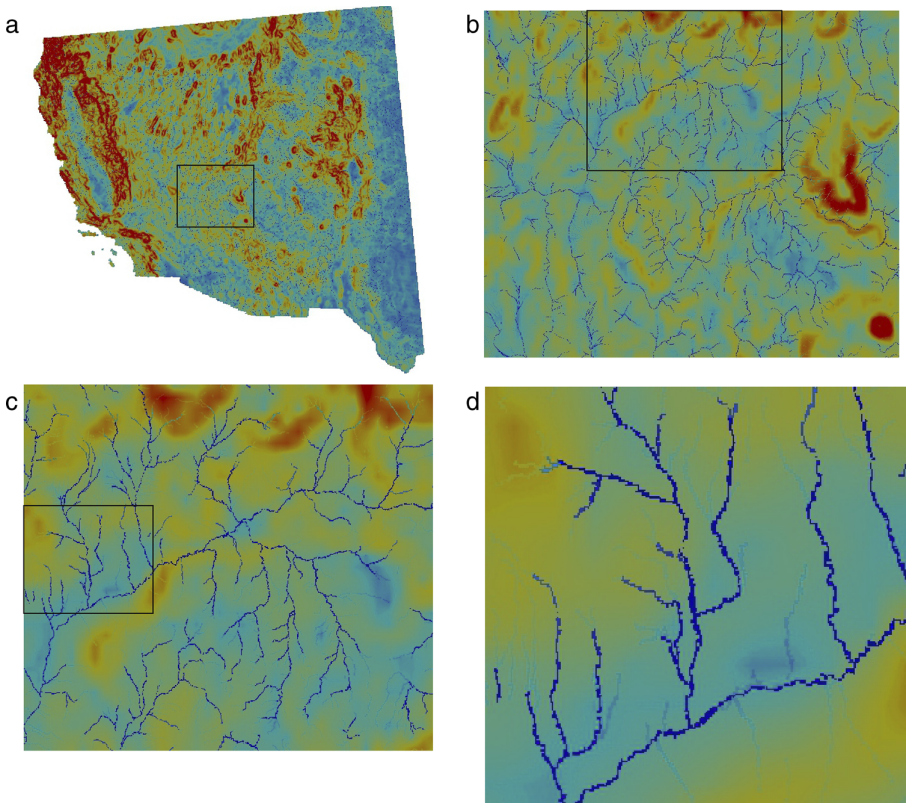

FIG. 6. Resistance map produced by the optimization of the relative influences of river network and variation in seasonal precipitation on gene flow of Fremont cottonwood. Resistance increases from a minimum of 1 in dark blue areas (rivers of orders 5, 6, and 7 in regions with little gradient in winter–spring precipitation), to a maximum of 18.97 in dark red areas (terrestrial uplands in regions with steep gradients of change in winter–spring precipitation. (a) The full study area extent is shown, (b) shows the window indicated by the box in panel (a), (c) the window indicated by the box in panel (b), and (c) the window indicated by the box in panel (c).

to large rivers equally facilitate gene flow, while resistance increases greatly as stream size becomes smaller. One possible explanation for this pattern of change in resistance with river order is that populations will have historically been larger and more connected along large rivers than smaller rivers, and that larger rivers provide better means for hydrological seed transport, and better conditions for seed germination and seedling establishment on sandbars. (Braatne et al. 1996, Rood et al. 2005)

Climate gradients and gene flow

Our second hypothesis proposed that genetic structure would be partly driven by climatic gradients. Consistent with this hypothesis, genetic differentiation increased with cumulative differences in winter and spring precipitation. We found no support for genetic differentiation based on differences between climatic zones or point climatic conditions at the sites of populations, but strong support increased genetic differentiation as a function of cumulative difference in winter and spring precipitation between populations. This suggests that seasonal differences in precipitation result in reduced gene flow, plausibly due to the effects on flowering phenology. It is important to note, however, that winter and spring precipitation can correlate with temperature and day length, which are additional climate variables that we did not investigate. Thus, the differentiation we observed based on precipitation alone could be part of multivariable interaction that includes these additional variables.

Our results suggest that genetic differences among cottonwood populations increase cumulatively as a function of climatic differences. The most supported climate model was path based, and not point based. Point-based genetic differences might be expected if certain genetic characteristics were found in certain environments, and were not dependent on patterns of population connectivity and gene flow. In contrast, path-based genetic differentiation, as found here, would be expected when gene flow is cumulatively reduced along paths between populations.

Adaptation to local environments can be a major driver of population divergence (Wright 1932, McKin-

non et al. 2004, Savolainen et al. 2007). Such ecological-based divergence has been shown to be plausible even in the presence of gene flow (Gavrilets et al. 2000, Niemiller et al. 2008, Nosil 2008). One of the major drivers of ecological divergence is differential timing of reproductive events (Feder et al. 1993, Yamamoto and Sota 2009). This suggests that gradients of rapid change in winter and spring precipitation may have acted as highly resistant zones that create attenuated gene flow, enabling genetic differentiation of populations. Our results are consistent with the hypothesis that differences in flowering phenology along gradients of changing winter and spring precipitation will influence pollination and seed dispersal and/or establishment, and drive differential patterns of gene flow. These differences could lead to sufficient reduction in gene flow, which may ultimately enable speciation due to accumulation of genetic incompatibilities (Gavrilets et al. 2000, Hoelzer et al. 2008).

In addition, the differential climatic conditions across the precipitation gradient likely impose directional selection on local populations. These climatic differences could reduce fitness of maladapted individuals, resulting in population divergence and maintenance of reproductive isolation (Gavrilets et al. 2000, 2007, Niemiller et al. 2008, Nosil 2008, de León et al. 2010). Yang et al. (2013) found a similar pattern of genetic differentiation along gradients of winter precipitation for shrub taxa in China, which they hypothesized was a result of partial reproductive isolation due to timing of pollen dispersal coupled with directional selection driven by drought tolerance. A similar combination of factors may be responsible for the observed genetic differentiation of Fremont cottonwood along climatic gradients. It is likely that a combination of reduced gene flow driven by differential timing of reproduction due to differences in seasonal precipitation patterns (Feder et al. 1993, Yamamoto and Sota 2009) in conjunction with local directional selection (Niemiller et al. 2008, Nosil 2008) and connectivity of the river network led to population differentiation of Fremont cottonwood across the southwest.

Our modeling results do not support isolation by distance as a significant factor in determining genetic differentiation in Fremont cottonwood. When optimized models of river, upland, and climate gradient resistance to gene flow were combined, there was no residual support for null models of isolation by distance or isolation by STRUCTURE clustering groupings. We treated isolation by distance and isolation by STRUCTURE clustering groupings as null models in this analysis, and our finding that there is no independent support for them confirms our expectation that correlations with these null models are spurious (Cushman and Landguth 2010). Isolation by distance would be expected when there is no differential gene flow related to landscape features. However, our model optimization clearly showed that both climate gradients and river network connectivity are highly related to genetic differentiation in Fremont cottonwood. Once the effects of river network and climate gradients are taken into account, there was no independent relationship with distance. In addition, STRUCTURE clustering identifies grouping of genetically similar populations without any a priori hypotheses of driving factors. Given they lack any a priori basis, these clusters are observations of differentiation and not explanations. As we expected, once the effects of river network connectivity and climate gradients are accounted for, there was no independent support for STRUCTURE clusters of genetically similar populations.

By combining restricted model optimization (Shirk et al. 2010) with reciprocal causal modeling (Cushman et al. 2013), we found that river network connectivity and climate gradients drive gene flow of Fremont cottonwood, and identify optimized resistance parameters for each landscape feature. In each step of the optimization, reciprocal causal modeling effectively identified a single candidate model that was independently supported relative to the other candidate models. This is a large improvement over previous model selection methods in landscape genetics, which typically struggle with high levels of Type I error and discriminating among multiple supported models (Cushman and Landguth 2010).

Conservation implications

Our study demonstrates that riparian corridors, in conjunction with seasonal differences in precipitation, facilitate gene flow in Fremont cottonwood, while terrestrial uplands constrain it. These results have consequences for maintaining genetic diversity, which impacts both riparian biodiversity and ecosystem processes. For example, recent studies of intraspecific variation in cottonwoods have shown that genetic diversity, arising from gene flow among populations, is linked to community composition and diversity (Ferrier et al. 2012, Bangert et al. 2013, Busby et al. 2013), community stability (Keith et al. 2010), nutrient cycling (Schweitzer et al. 2008), and productivity (Grady et al. 2011). These genetics-based effects on community structure and ecosystem processes are commonly observed worldwide (Whitham et al. 2012), but are rarely incorporated into management strategies. We argue that the maintenance of riparian corridors that facilitate gene flow and generate genetic diversity is critical for the preservation of biological diversity in riparian ecosystems.

Because most arid lands riparian systems are threatened by habitat loss, invasive species, water diversions and altered stream flows (Noss et al. 1995, Friedman et al. 2005, Rood et al. 2005), increased habitat fragmentation and reduced gene flow threaten the plants that support much larger communities of organisms and their ecosystem process. Thus, conservation efforts should focus not only on restoring riparian habitat, as in the case of large-scale efforts underway for targeted

areas in the southwestern United States (Lower Colorado River Multi-Species Conservation Program [LCR MSCP 2004]), but also on re-establishing corridors that promote gene flow. Given that community structure in cottonwoods scales from local (Ferrier et al. 2012, Bangert et al. 2013) to regional levels (Bangert et al. 2008), it is important to consider how gene flow across the landscape (e.g., geographic mosaic theory; Thompson 2005) may influence the evolution of dependent community members (e.g., Evans et al. 2008), and affect community diversity and ecosystem processes (Allan et al. 2012).

In addition to habitat fragmentation, our results relate to adaptation to potential impacts of climate change (e.g., Aitken et al. 2008). Because Fremont cottonwood is known to be sensitive to climate (Grady et al. 2011) and invasive species (Gitlin et al. 2006), which may then act in concert to further the demise of a species (Walther et al. 2009), it is important that we understand the nature and extent of causal factors that influence patterns of gene flow and structure in this foundation tree species. Given that gene flow appears to be jointly driven by river corridor connectivity and climatic differences, habitat loss, coupled with climate change, is likely to fragment populations that are currently along major river courses. Such fragmentation could result in reduced gene flow along riparian corridors, leading to increased inbreeding within populations. If fragmentation were extensive, smaller populations would be vulnerable to genetic drift, and the deleterious effects of inbreeding depression. In the face of climate change, these effects could limit individual populations' ability to adapt to a changing environment and ultimately result in the loss of genetic diversity. By understanding the landscape and environmental features that determine gene flow and genetic differentiation, conservationists can more efficiently manage species such as Fremont cottonwood, while at the same time ensuring support of its dependent communities and associated ecosystem processes.

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Literature Cited

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang, and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: Climate change outcomes for tree populations. Evolutionary Applications 1:95–111.

Allan, G. J., S. Shuster, S. Woolbright, F. Walker, N. Meneses, A. Keith, J. Bailey, and T. G. Whitham. 2012. Interspecific indirect genetic effects (IIGEs): Linking genetics and genomics to community ecology and ecosystem processes. Pages 295–323 *in* T. Ohgushi, O. Schmitz, and R. D. Holt, editors. Ecology and evolution of trait-mediated indirect interactions: linking evolution, community, and ecosystems. Cambridge University Press, Cambridge, UK.

Amos, J., A. F. Bennet, R. Mac Nally, G. Newell, J. Q. Radford, A. Pavlova, J. Thompson, M. White, and P. Sunnucks. 2012. Predicting landscape genetic consequences of habitat loss, fragmentation and mobility for species of woodland birds. PLoS ONE 7:e30888.

Applied Biosystems. 2011. GeneMapper version 4.0. Thermo Fischer Scientific, Waltham, Massachusetts, USA.

Bangert, R., L. Evans, S. Ferrier, G. J. Allan, and T. G. Whitham. 2013. The proportion of three foundation plant species and their genotypes influence an arthropod community: restoration implications for the endangered southwestern willow flycatcher. Restoration Ecology 21:44–7456.

Bangert, R. K., E. V. Lonsdorf, G. M. Wimp, S. M. Shuster, D. Fischer, J. A. Schweitzer, G. J. Allan, J. K. Bailey, and T. G. Whitham. 2008. Genetic structure of a foundation species: scaling community phenotypes from the individual to the region. Heredity 100:121–131.

Braatne, J. H., S. B. Rood, and P. E. Heilman. 1996. Life history, ecology, and reproduction of riparian cottonwoods in North America. Pages 57–85 *in* R. F. Stettler, H. D. Bradshaw, P. E. Heilman, and T. M. Hinckley, editors. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Canada.

Burczyk, J., S. P. DiFazio, and W. T. Adams. 2004. Gene flow in forest tress: how far do genes really travel? Forest Genetics 11(3–4):179–192.

Busby, P. E., G. Newcombe, R. Dirzo, and T. G. Whitham. 2013. Genetic basis of pathogen community structure for foundation tree species in a common garden and in the wild. Journal of Ecology 101:867–877.

Castellano, S., and E. Balletto. 2002. Is the partial Mantel test inadequate? Evolution 56:1871–1873.

Coulon, A., J.-F. Cosson, M. A. Angibault, S. Aulagnier, B. Cargnelutti, M. Galan, and J. M. Hewison. 2004. Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. Molecular Ecology 13:2841–2850.

Cushman, S. A., and E. L. Landguth. 2010. Spurious correlations and inference in landscape genetics. Molecular Ecology 19:3592–3602.

Cushman, S. A., T. N. Wasserman, E. L. Landguth, and A. J. Shirk. 2013. Re-evaluating causal modeling with Mantel tests in landscape genetics. Diversity 5:51–72.

de León, L. F., E. Bermingham, J. Podos, and A. P. Hendry. 2010. Divergence with gene flow as facilitated by ecological differences: within-island variation in Darwin's finches. Philosophical Transactions of the Royal Society B 365:1041–1052.

Earl, D. A., and B. M. vonHoldt. 2011. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Version v0.6.8. Conservation Genetics Resources 4:359–361.

Eckenwalder, J. 1977. North American cottonwoods (*Populus*, Salicaceae) of sections Abaso and Aigeros. Journal of the Arnold Arboretum 58:193–208.

Eckenwalder, J. 1984. Natural intersectional hybridization between North American species of *Populus* (Salicaceae) in sections Aigeiros and Tacamahaca. I. Population studies of *P.* × *parryi*. Canadian Journal of Botany 62:317–324.

Ellison, A. M., et al. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment 3:479–486.

ESRI. 2011. ArcGIS Desktop: Release 10. Environmental Systems Research Institute, Redlands, California, USA.

ESRI. 2012. ArcGIS spatial analyst. Environmental Systems Research Institute, Redlands, California, USA.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software Structure: A simulation study. Molecular Ecology 14:2611–2620.

Evans, L. M., G. J. Allan, S. M. Shuster, S. A. Woolbright, and T. G. Whitham. 2008. Tree hybridization and genotypic variation drive cryptic speciation of a specialist mite herbivore. Evolution 62:3027–3040.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–87.

Feder, J. L., T. A. Hunt, and L. Bush. 1993. The effects of climate, host plant phenology and host fidelity on the genetics of apple and hawthorn infesting races of *Rhagoletis pomonella*. Entomologia Experimentalis et Applicata 69:117–135.

Ferrier, S., R. K. Bangert, E. I. Hersch-Green, J. K. Bailey, G. J. Allan, and T. G. Whitham. 2012. Unique arthropod communities on different host-plant genotypes results in greater arthropod diversity. Arthropod Plant Interactions 6:187–195.

Follstad Shah, J. J., C. N. Dahm, S. P. Gloss, and E. S. Bernhardt. 2007. River and riparian restoration in the Southwest: results of the National River Restoration Science Synthesis Project. Restoration Ecology 15:550–562.

Friedman, J. M., G. T. Auble, P. B. Shafroth, M. L. Scott, M. F. Merigliano, M. D. Freehling, and E. R. Griffin. 2005. Dominance of non-native riparian trees in western USA. Biological Invasions 7:747–751.

Gavrilets, S., H. Li, and M. D. Vose. 2000. Patterns of parapatric speciation. Evolution 54:1126–559. 1134.

Gavrilets, S., A. Vose, M. Barluenga, W. Salzburger, and A. Meyer. 2007. Case studies and mathematical models of ecological speciation. 1. Cichlids in a crater lake. Molecular Ecology 16:2893–2909.

Gitlin, A. R., C. M. Sthultz, M. A. Bowker, S. Stumpf, K. L. Paxton, K. Kennedy, A. Munoz, J. K. Bailey, and T. G. Whitham. 2006. Mortality gradients within and among dominant plant populations as barometers of ecosystem change during extreme drought. Conservation Biology 20:1477–1486.

Gleyzer, A., M. Denisyuk, A. Rimmer, and Y. Salingar. 2004. A fast recursive GIS algorithm for computing Strahler stream order in braided and nonbraided networks. Journal of the American Water Resources Association 40:937–946.

Grady, K. C., S. M. Ferrier, T. G. Whitham, T. E. Kolb, S. C. Hart, and G. J. Allan. 2011. Genetic variation in productivity of foundation riparian species at the edge of their distribution: implications for restoration and assisted migration in a warming climate. Global Change Biology 17:3724–3735.

Guillot, G., and F. Rousset. 2011. On the use of simple and partial Mantel tests in the presence of spatial autocorrelation. Methods in Ecology and Evolution 4:336–344.

Hoelzer, G. A., R. Drewes, J. Meier, and R. Doursat. 2008. Isolation-by-distance and outbreeding depression are sufficient to drive parapatric speciation in the absence of environmental influences. PLoS Computational Biology 4:e1000126.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806.

Keith, A. R., J. K. Bailey, and T. G. Whitham. 2010. A genetic basis to community repeatability and stability. Ecology 11:3398–3406.

Landguth, E. L., and S. A. Cushman. 2010. CDPOP: a spatially explicit cost distance population genetics program. Molecular Ecology Resources 10:156–161.

Landguth, E. L., N. Johnson, and S. A. Cushman. 2011. Simulating selection in landscape genetics. Molecular Ecology Resources 12:363–386.

Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology 74:1659–1673.

Legendre, P., and M.-J. Fortin. 2010. Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. Molecular Ecology Resources 10:831–844.

Legendre, P., and M. Troussellier. 1988. Aquatic heterotrophic bacteria: modeling in the presence of spatial autocorrelation. Limnology and Oceanography 33:1055–1067.

LeRoy, C. J., T. G. Whitham, P. Keim, and J. C. Marks. 2006. Plant genes link forests and streams. Ecology 87:255–261.

LCR MSCP [Lower Colorado River Multi-species Conservation Program]. 2010. Lower Colorado River Multi-species Conservation Program Volume II: habitat conservation plan. Final. LCR MSCP, Sacramento, California, USA.

Little, E. L., Jr., 1971. Atlas of United States trees. Volume 1, conifers and important hardwoods. Miscellaneous publication 1146. U.S. Department of Agriculture, Washington, D.C., USA.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

McKinnon, J. S., S. Mori, B. K. Blackman, L. David, D. M. Kingsley, L. Jamieson, J. Chou, and D. Schluter. 2004. Evidence for ecology's role in speciation. Nature 429:294–298.

McRae, B. H. 2006. Isolation by resistance. Evolution 60:1551–1561.

Meirmans, P. G. The trouble with isolation by distance. 2012. Molecular Ecology 21:2839–2846.

Niemiller, M. L., B. M. Fitzpatrick, and B. T. Miller. 2008. Recent divergence-with-gene-flow in Tennessee cave salamanders (Plethodontidae; Gyrinophylus) inferred from gene genealogies. Molecular Ecology 17:2258–2275.

Nosil, P. 2008. Speciation with gene flow could be common. Molecular Ecology 17:2103–2106.

Noss, R. F., E. T. LaRoe, and J. M. Scott. 1995. Endangered ecosystems of the United States: A preliminary assessment of the loss and degradation. Biological report 28. U.S. Department of the Interior, Washington, D.C., USA.

Peakall, R., and P. E. Smouse. 2006. GenAlEx v. 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–59.

Raufaste, N., and F. Rousset. 2001. Are partial Mantel tests adequate? Evolution 55:1703–1705.

Rood, S. B., G. M. Samuelson, J. H. Braatne, C. R. Gourley, F. M. R. Hughes, and J. M. Mahoney. 2005. Managing river flows to restore floodplain forests. Frontiers in Ecology and Environment 3:193–201.

Rosenberg, N. A. 2004. Distruct: a program for the graphical display of population structure. Molecular Ecology Notes 4:137–138.

Savolainen, O., T. Pyhäjärvi, and T. Knürr. 2007. Gene flow and local adaptation in trees. Annual Review of Ecology, Evolution, and Systematics 38:595–619.

Schwartz, M. K., J. P. Copeland, N. J. Anderson, J. R. Squires, R. M. Inman, K. S. McKelvey, K. L. Pilgrim, L. P. Waits, and S. A. Cushman. 2009. Wolverine gene flow across a narrow climatic niche. Ecology 90:3222–3232.

Schweitzer, J. A., et al. 2008. From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. Ecosystems 11:1005–1020.

Shirk, A., D. O. Wallin, S. A. Cushman, R. C. Rice, and C. Warheit. 2010. Inferring landscape effects on gene flow: a new multi-scale model selection framework. Molecular Ecology 19:3603–1619.

Shuster, S. M., E. V. Lonsdorf, G. M. Wimp, J. K. Bailey, and T. G. Whitham. 2006. Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. Evolution 60:991–1003.

Slavov, G. T., S. Leonardi, J. Burczyk, W. T. Adams, S. H. Strauss, and S. P. DiFazio. 2009. Extensive pollen flow in two ecologically contrasting populations of *Populus trichocarpa*. Molecular Ecology 18:357–373.

Slavov, G. T., and P. Zhelev. 2010. Salient biological features, systematics and genetic variation of *Populus*. Pages 15–38 *in* S. Jansson, R. Bhalerao, and A. T. Groover, editors. Genetics and genomics of *Populus*. Spring, New York, New York, USA.

Smouse, P. E., J. C. Long, and R. R. Sokal. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Systematic Zoology 35:627–632.

Strahler, A. N. 1957. Quantitative analysis of watershed geomorphology. American Geophysical Union Transactions 38:913–920.

Thompson, J. N. 2005. The geographic mosaic of coevolution. University of Chicago Press, Chicago, Illinois, USA.

Tuskan, G. A., L. E. Gunter, Z. K. Yang, T. M. Yin, M. M. Sewell, et al. 2004. Characterization of microsatellites revealed by genomic sequencing of *Populus trichocarpa*. Canadian Journal of Forest Research 34:85–93.

Walther, G., et al. 2009. Alien species in a warmer world: risks and opportunities. Trends in Ecology and Evolution 24:686–693.

Wasserman, T. N., S. A. Cushman, M. K. Schwartz, and D. O. Wallin. 2010. Spatial scaling and multi-model inference in landscape genetics: *Martes americana* in northern Idaho. Landscape Ecology 25:1601–1612.

Wasserman, T. N., S. A. Cushman, A. S. Shirk, E. L. Landugth, and J. S. Littell. 2012. Simulating the effects of climate change on population connectivity of American marten (*Martes americana*) in the northern Rocky Mountains, USA. Landscape Ecology 27:211–225.

Whitham, T. G., J. K. Bailey, J. A. Schweitzer, S. M. Shuster, R. K. Bangert, C. J. LeRoy, E. V. Lonsdorf, G. J. Allan, S. P. DiFazio, and B. M. Potts. 2006. A framework for community and ecosystem genetics: From genes to ecosystems. Nature Reviews Genetics 7:510–523.

Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding and selection in evolution. Pages 356–366 *in* Proceedings of the sixth international congress of genetics. http://www.esp.org/books/6th-congress/facsimile/contents/6th-cong-p356-wright.pdf

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420.

Yamamoto, S., and T. Sota. 2009. Incipient allochronic speciation by climatic disruption of the reproductive period. Proceedings of the Royal Society B 276:2711–2719.

Yang, J., S. A. Cushman, J. Yang, M. Yang, and T. Bao. 2013. Effects of climatic gradients on genetic differentiation of *Caragana* on the Ordos Plateau, China. Landscape Ecology 28:1729–1741.

Supplemental Material

Appendix A

Tables listing the locations of sampled populations and the characteristics of the SSR primers used for genetic analysis (*Ecological Archives* A024-059-A1).

Appendix B

Figures showing the results of the intermediate steps of the reciprocal causal modeling analysis to optimize relative resistance to gene flow presented by rivers and climate gradients (*Ecological Archives* A024-059-A2).