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Original Article

Rivers as barriers to gene flow: A comparative population genomic analysis using causal modeling

Garett L. Maag[1], Austin R. Biddy[2], Maya F. Stokes[3], Greer A. Dolby[2,*]

[1]School of Life Sciences, Arizona State University, Tempe, AZ, United States
[2]Department of Biology, University of Alabama at Birmingham, Birmingham, AL United States
[3]Department of Earth, Ocean & Atmospheric Science, Florida State University, Tallahassee, FL, United States
*Corresponding author: Department of Biology, 1720 2nd Ave S, University of Alabama at Birmingham, Birmingham, AL, 35294, United States.
Email: gdolby@uab.edu

Abstract

Earth's physiographic features shape the genetic evolution of organisms, but understanding how such features act as barriers to gene flow requires quantifying characteristics of both the barrier and the organism. Many barrier characteristics, however, are interdependent and not fully captured by traditional multivariate statistics. Here, we evaluate the use of directed acyclic (causal) graphs and structural equation modeling (SEM) to test the Riverine Barrier Hypothesis using 27 river-spanning population genomic datasets of terrestrial plants and animals associated with 24 rivers across the contiguous United States. These data were paired with seasonality, river width, and river discharge data. SEM analysis revealed patterns not captured by standard approaches. River width had a strong effect on population differentiation, with distinct direct and indirect effects for high and low dispersers. Results suggest a negative width-Fst relationship for low dispersers, which we interpret to be due to topographic context of higher elevation or bedrock-incised rivers. In contrast, high dispersers had a positive relationship, indicating wider rivers present a greater barrier to dispersal. The total effect of river discharge was negligible because its direct effects on population differentiation were canceled out by indirect effects on other river features. Overall, the best-fitting SEM explained 52% of population differentiation for low dispersers and 13% for high dispersers, consistent with the idea that high-dispersing species are less impacted by river barrier effects. This proof of concept shows the utility of causal graphs and SEM at modeling complex relationships between Earth's physiographic features and the organisms that evolve with them.

Keywords geogenomics, population structure, SEM, DAG, causality, rivers

Introduction

The riverine barrier hypothesis Wallace articulated in 1854 poses that rivers act as barriers that limit, reduce, or prevent migration (and therefore gene flow) among terrestrial populations (Wallace 1854). With gene flow reduced for enough time, allopatric speciation may occur, increasing species richness. This provided an early hypothesis for why species diversity is high in the Amazon—that it arose through allopatric speciation across large, impenetrable river channels (Colwell 2000; Musher 2025). Since then, many studies have tested this hypothesis across taxa, climates, geographies, and types of rivers using mark-recapture, species surveys, and (most commonly) molecular data to assess population structure (Ribas et al. 2025). Yet, results have been highly variable and at times directly conflicting (see Table 1 for a summary of some prior results), making the riverine barrier hypothesis a topic of great debate within comparative phylogeography and biogeography (Musher 2025). For example, on the Guiana Shield in the Amazon, Fouquet et al. (2015) surveyed frogs across habitat types and body sizes and found mixed results with significant river-associated divergence in about half (13 of 28) of species (Fouquet et al. 2015); those with positive relationships segregated at least in part by habitat and life history characteristics. Results from studies of river-related divergence have been mixed in primates (Jalil et al. 2008; Boubli et al. 2015; Link et al. 2015) and other amphibians (Gehring et al. 2012; Godinho and Da Silva 2018), but rivers are often *not* a barrier to small mammals (Klee et al. 2004; Colombi et al. 2010; Roratto et al. 2015).

Dispersal ability (and other life history traits) is often expected to mediate river effects, with more highly dispersive organisms predicted to be less strongly structured by rivers (Paz et al. 2015; Papadopoulou and Knowles 2016). Yet that is not always the case, at least for an Amazonian tree (Nazareno et al. 2019a) and an African bird (as well as its parasitic lice, Naka et al. 2022, but also see Musher et al. 2022; Oliveira et al. 2017). In China, Zhang et al. found that the Yangtze River restricts seed dispersal in a large shrub but not its pollen, which is lighter, leading to male-biased cross-river gene flow (Zhang et al. 2007). These selected examples

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Table 1 Illustrative summary of selected studies where the riverine barrier hypothesis was tested to show the diversity of results.

Reference	Taxon	Location	*N*	Result
Boubli et al. 2015	Primates	Brazil	10	
Cáceres 2007	Small mammal	Brazil	4	
Coelho et al. 2022	Lizards, frog, small mammal, bird	Brazil	6	
Colombi et al. 2010	Small mammal	Brazil	1	
Godinho and Da Silva 2018	Frogs	Amazonia	577	
Figueiredo-Vázquez et al. 2021	Salamanders	Iberian Peninsula	1	
Fouquet et al. 2015	Frogs	Guiana shield	28	
De Fraga and De Carvalho 2022	Frogs and reptiles	Brazil	89	
Funk et al. 2007	Frogs	Amazon basin	1	
Gascon et al. 1998	Frogs	Brazil	4	
Gascon et al. 2000	Frogs, small mammals	Brazil/Peru	>29	
Gehring et al. 2012	Amphibians	Madagascar	4	
Gonçalves-Sousa et al., 2022	Lizards	Brazil	63	
Jalil et al. 2008	Primates	Borneo	1	
Klee et al. 2004	Small mammal	US	1	
Kopuchian et al. 2020	Birds	Del Plata basin	7	
Link et al. 2015	Primates	Colombia	1	
Nazareno et al. 2017	Shrub	Brazil	1	
Nazareno et al. 2019b	Woody plants	Brazil	3	
Nazareno et al. 2019a	Tree	Brazil	1	
Patton et al. 1994	Small mammal	Amazonia	1	
Peres et al. 1996	Primate	Brazil	1	
Roratto et al. 2015	Small mammal	Brazil	1	
Townsend et al. 2009	Lizards	Madagascar	28	
Vieira et al. 2022	Insect	Brazil	14	
Voelker et al. 2013	Birds & lice	Congo basin	10	
Zhang et al. 2007	Plant	China	1	

Listed are the focal taxa, geographic location, and qualitative summary of findings. Number of taxa included in the study (some studies had many populations but only one species). These results are displayed qualitatively in the last column as the river was: red—a significant barrier, light purple—a low-level barrier, dark purple—results were mixed (positive and negative) by river or taxon, or green—not a barrier. This is not an exhaustive list of studies and these were not the datasets used in the SEM analysis.

highlight that dispersal ability does not fully explain when river barriers affect species (Ribas et al. 2025), suggesting aspects of these systems have not yet been sufficiently captured.

While organismal characteristics have been considered, there has been comparably little attention paid to characterizing the rivers themselves (but see Jackson and Austin 2013; Dolby et al. 2019; Ruokolainen et al. 2019; Ribas et al. 2022, 2025; Musher 2025). Larger river channels should serve as stronger barriers than smaller branches or tributaries, which was observed in a shrub species in Amazonia (Nazareno et al. 2017). Accordingly, some have predicted wider river mouths are stronger barriers than narrower headwaters, but this has received mixed support (Patton et al. 1994; Peres et al. 1996; Godinho and Da Silva 2018; Figueiredo-Vázquez et al. 2021) and might depend on discharge, sediment load and channel complexity, or on associated habitat. Conversely, it could be argued that although rivers at high elevations tend to be narrower, they often have greater cross-valley topographic relief and these elevation changes can restrict dispersal more than the flatter, low-elevation landscapes where rivers tend to be wider. Indeed many aspects of rivers vary from up to downstream, including their slope, width, water discharge, and flow characteristics (Leopold and Maddock 1953; Leopold et al. 1964; Park 1977). Although these variables are interrelated, each may play its own distinct role in barrier effects. Similarly, a history of meander cut-offs, avulsions, or stream capture may influence historic connectivity across contemporary barriers (Stokes et al. 2023; Musher 2025). It is therefore possible that the mixed support for the riverine barrier hypothesis is simply a sampling artifact of studying many different types of organisms over many different types of river settings without fully accounting for the attributes of both (Paz et al. 2015; Musher 2025; Schrodt et al. 2025).

Resolving the above question requires three analytical advances. First, aspects of the proposed river barrier must be sufficiently quantified in a standardized way to allow direct comparison both within and across different rivers. Second, the statistical interdependency of the riverine characteristics must be appropriately considered. Third, the statistical framework must integrate primary riverine and population divergence data so the results

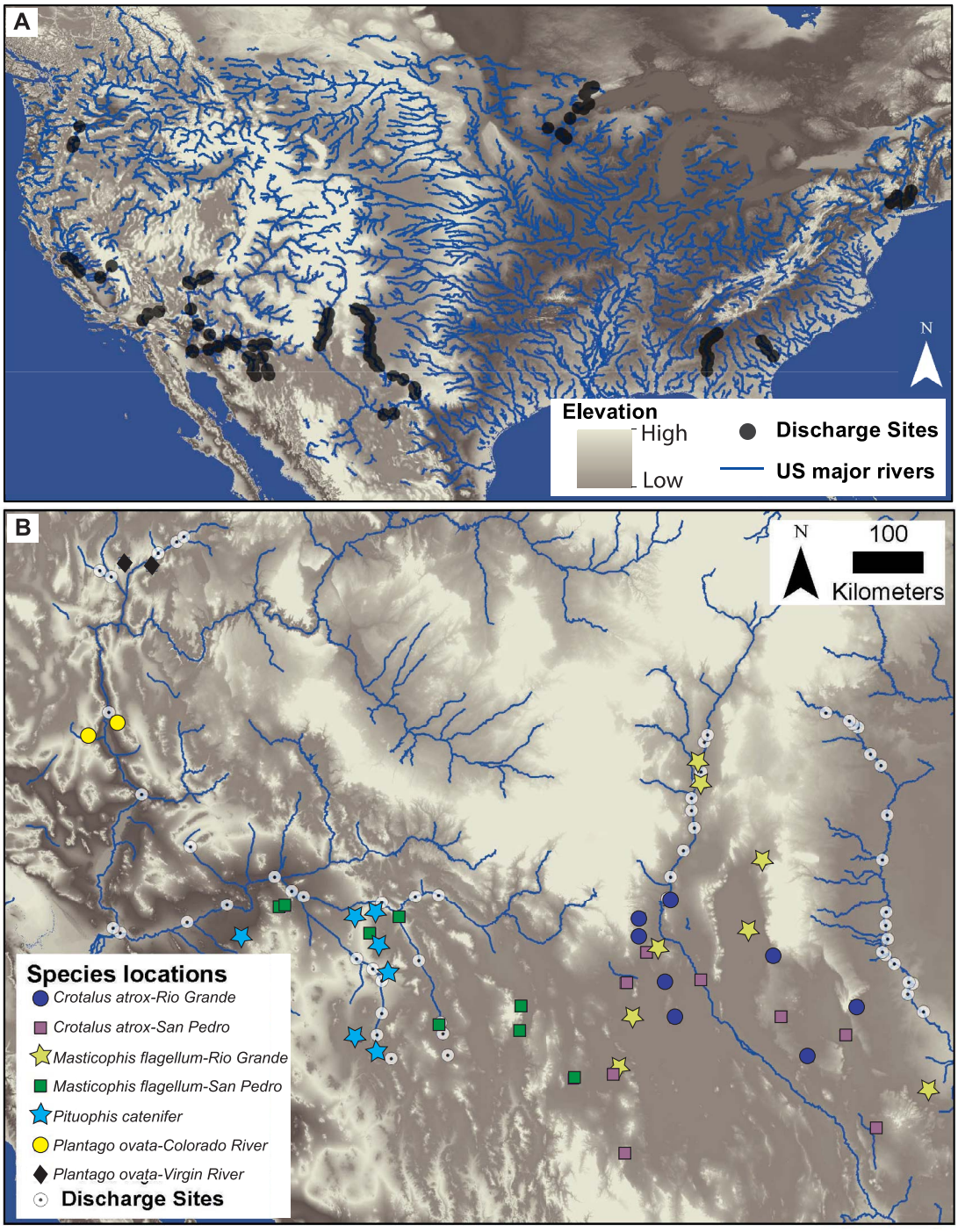


Fig. 1 Map of study area and river networks. (A) Locations of rivers sampled in this study are shown by discharge sites (gauges) with SRTM 90-m resolution digital elevation model as a base map. (B) An example set of rivers from the southwest with associated individuals within population pairs (colored shapes) and discharge sites. River width was calculated at five locations near each discharge site and averaged. Not all species in this area are plotted to ease visualization.

from one study system can be directly compared with another, limiting study-to-study noise (batch effects) and allowing for development of a more robust working theory (Schrodt et al. 2025). Rather than asking, "*Do* rivers structure populations?", capturing complexities of both the river and the organism will allow us to ask the more nuanced question, "*Under what conditions* do rivers structure populations, and among which species?".

In this study, we aimed to satisfy these requirements and evaluate the riverine barrier hypothesis by analyzing genomic data from published literature for 27 population pairs of terrestrial plant and animal species traversing 24 river systems across the contiguous United States (Fig. 1). We obtained river discharge, discharge seasonality, and river width measurements for locations where population genomic data spanning a river channel was available. We first used traditional multivariate statistical techniques to assess

Table 2 Description and justification of pathways used to build the DAGs that were tested (DAG1–3; Figs. 2–5). Observed relationship (Obs.) gives the observed relationship across all analyses.

#	Pathway	Expected relationship	Obs.	Justification of path
1	Discharge → seasonality	Negative	Yes	Higher discharge associated with larger catchment, larger catchment is less susceptible to seasonal fluctuations
2	Discharge → river width	Positive	∼Yes	Higher water discharge associated with wider channels, empirically confirmed for rivers across the US and globally
3	Discharge → Fst	Positive	∼Yes	Higher flow would be harder for organisms to swim or drift across
4	River width → Fst	Positive	∼No	Wider rivers would be harder for an organism to swim or drift across
5	Seasonality → Fst	Negative	∼No	High seasonality implies periods of low flow where the river may be easy to cross
6	River width → geographic distance	Positive	No	Wider rivers have banks farther apart, therefore absolute distance must be greater than river distance
7	Geographic distance → Fst	Positive	No	Isolation by distance principle, it is easier to disperse locally than farther away
8	Dispersal → Fst	Negative	∼Yes	An organism that can move farther distances can traverse a water barrier easier than one that cannot

Tilde means the answer is qualified. Note that the *total* indirect path from width to distance to Fst is positive for low dispersers.

which river characteristic(s), if any, explain genetic differentiation among organisms and then compared these to results from a graph-based statistical approach, structural equation modeling (SEM), to see what (if anything) can be additionally learned from this approach. SEM is based on directed acyclic graphs (DAGs) that capture known direct and indirect relationships (i.e. pathways; Table 2) among variables (Supplementary Fig. S1). In this case, known relationships among river attributes from theory and empirical literature were drawn and their individual and interactive controls on genomic differentiation (as Fst) were assessed.

Materials & methods

Curation of genomic data

We mined published literature using combinations of keywords in Google Scholar and Web of Science including: "river", "riverine barrier", "river barrier hypothesis", "population structure", "speciation", "phylogeography", "genome", "whole genome". From these, we selected studies that contained population genomic datasets that: (1) were publicly accessible, (2) provided Global Positioning System (GPS) coordinates for sampled individuals, (3) contained at least three individuals sampled on each side of a river (see Table 3 for data sources). Only terrestrial species were chosen for analyses as aquatic, semiaquatic, or amphibious species can disperse within river systems and river characteristics may affect their population structure in different ways. Datasets used either whole genome sequencing (WGS) or Restriction-Site Associated DNA sequencing (RAD-seq). The original studies did not necessarily generate data to study riverine barriers so the datasets may reflect other evolutionary patterns that could increase variance in our models, which we partly control for by evaluating population structure of each dataset. For each paper, we recorded the genus and species, organism group, accession numbers, GPS coordinates, and type of genomic data (WGS or RAD-seq). The final dataset included birds, herbs, lizards, rodents, snakes, and trees (for a list of taxa see Table 3).

Assessing population structure

Because river-associated genetic differentiation can be obscured by other population structuring or admixture, we used Structure v.2.3.4 (Falush et al. 2007) to quality control the data by testing for population structure in each population pair. Specifically, we sought to omit individuals from datasets for which a $K = 3$ was favored on the premise that high population structure on one side of a river (not due to the river) would deflate or obscure cross-river F_{ST} values and would reflect alternate evolutionary processes that were not the focus here. We used PGDSpider (Lischer & Excoffier 2011) to convert VCF files to Structure format. If the data were in a multi-locus FASTA format, we used Fasta2Structure (Bessa-Silva 2024) for file conversion. We ran Structure for each dataset for 100,000 Markov Chain Monte Carlo (MCMC) replications with a burn-in of 5,000 for K values 1 to 3, and three replicates per K per dataset. To summarize across replicates, we used structure*H*arvester v0.7 (Earl & vonHoldt 2011) to compile the outputs and determine the favored K for each dataset based on the lowest mean log likelihood. If a dataset favored $K = 3$, individuals with ancestry primarily from a third population were removed from that dataset; after removal of these individuals, datasets that did not meet our criteria (minimum three individuals per population) were removed from further analysis. We note studies seeking to understand the full suite of evolutionary processes shaping population structure should retain admixed individuals.

Justification for sampling design

Traditional phylogeographic studies often sample broadly across a species' range to identify the primary features structuring populations through statistically supported descriptive clustering. In this study, however, we address a fundamentally different question—estimating the specific contribution of river barrier attributes to genetic differentiation across diverse species. This requires restricting geographic sampling within each dataset to areas immediately adjacent to the river segment so SEM can

Table 3 Information for the 27 genomic datasets used in this study, including associated references, sample sizes (*N*), associated river, and data type (GBS, RADseq).

Group	Genus	Species	River	*N*	Removed	Fst	Data type	Reference
Snake	*Rhinochelius*	*lecontei*	Pecos	6	–	0.10	WGS	Myers et al. 2019
Snake	*Arizona*	*elegans*	Colorado	7	–	0.09	WGS	Myers et al. 2019
Snake	*Masticophis*	*flagellum*	Rio Grande	8	–	0.03	WGS	Myers et al. 2019
Snake	*Masticophis*	*flagellum*	San Pedro	8	–	0.40	WGS	Myers et al. 2019
Snake	*Crotalus*	*scutulatus*	Rio Grande	7	–	0.04	WGS	Myers et al. 2019
Snake	*Pituophis*	*catenifer*	Rio Grande	8	–	0.07	WGS	Myers et al. 2019
Snake	*Pituophis*	*catenifer*	San Pedro	6	–	0.00	WGS	Myers et al. 2019
Snake	*Crotalus*	*atrox*	Gila	8	–	0.05	WGS	Myers et al. 2019
Snake	*Crotalus*	*atrox*	San Pedro	8	–	0.04	WGS	Myers et al. 2019
Snake	*Crotalus*	*atrox*	Rio Grande	8	–	0.02	WGS	Myers et al. 2019
Snake	*Crotalus*	*atrox*	Santa Cruz	8	–	0.02	WGS	Myers et al. 2019
Lizard[a]	*Anolis*	*carolinensis*	Savannah	7	All	0.04	RAD-seq	Manthey et al. 2016
Rodent	*Peromyscus*	*leucopus*	Housatonic	15	–	0.12	WGS	Munshi-South et al. 2016
Rodent	*Peromyscus*	*leucopus*	Wallkill	17	–	0.02	WGS	Munshi-South et al. 2016
Rodent	*Peromyscus*	*leucopus*	Croton	15	–	0.06	WGS	Munshi-South et al. 2016
Herb	*Plantago*	*ovata*	Mojave	16	8	0.04	RAD-seq	Shryock et al. 2021
Herb	*Plantago*	*ovata*	Colorado	23	–	0.01	RAD-seq	Shryock et al. 2021
Herb	*Plantago*	*ovata*	Muddy	24	–	0.09	RAD-seq	Shryock et al. 2021
Herb	*Plantago*	*ovata*	Virgin	24	–	0.05	RAD-seq	Shryock et al. 2021
Tree	*Quercus*	*rubra*	Kettle	25	–	0.05	RAD-seq	Gomez Quijano et al. 2020
Tree	*Quercus*	*ellipsoidalis*	Mississippi	24	–	0.13	RAD-seq	Gomez Quijano et al. 2020
Tree	*Quercus*	*rubra*	St. Louis	28	–	0.08	RAD-seq	Gomez Quijano et al. 2020
Tree	*Quercus*	*rubra*	Knife	24	–	0.07	RAD-seq	Gomez Quijano et al. 2020
Tree	*Quercus*	*rubra*	Nemadji	26	–	0.13	RAD-seq	Gomez Quijano et al. 2020
Tree	*Quercus*	*rubra*	Kawaishiwi	24	–	0.28	RAD-seq	Gomez Quijano et al. 2020
Bird	*Junco*	*h. oreganus*	Deschutes	6	–	0.00	WGS	Friis et al. 2018
Bird	*Junco*	*h. oreganus*	San Joaquin	16	–	0.01	WGS	Friis et al. 2018

Fst refers to the pairwise Fst calculated in this study for cross-river populations. GBS, genotype by sequencing, RADseq, restriction associated DNA. [a] Lizard *Anolis carolinensis* was removed from analysis following data curation.

isolate barrier effects from other sources of variation. It also necessitates including datasets with unstructured populations in which the river is not an influential feature, as unstructured populations provide biologically meaningful counter-evidence for testing riverine barrier effects and avoids ascertainment bias. Therefore, our sampling design is intentionally optimized for the statistical requirements of SEM and differs from conventional phylogeographic practice by design. We could instead ask, "For species whose populations are structured by rivers, what aspect(s) of rivers are barriers" and in that case unstructured populations should be excluded.

Calculation of Fst

We calculated Fst using either the VCF file or multiFasta sequences provided by the original study and therefore relied on the original read processing, filtering, mapping, and variant calling used therein. Although this could affect Fst calculations, we expect the effects to be idiosyncratic and therefore not bias SEM results. Files were imported using the read.multiFASTA() function from the apex package (v1.0.4, Jombart et al. 2017). All files were converted to genind files using vcfR package (v1.12.0, Knaus and Grünwald 2017) in R Studio (v.1.3.1073, R Core Team 2021). Following our assessment of population structure, individuals closest to the river on each side were grouped (minimum $N = 3$ per side) and called a population. Small sizes can increase uncertainty in estimates of differentiation, though genome-wide estimates of Fst perform well with a sample size of five (Goudet and Weir 2023). Only three of the 27 river comparisons were smaller than this, while the rest were well above. We retained these comparisons because replicates across rivers and taxa is central to the comparative SEM framework and were unlikely to bias the overall SEM models. Each dataset contained two populations: one on each side of the river segment. The genind file was converted to a heirfstat file using the heirfstat package (v0.5-11, Goudet 2005) and a pairwise Fst value was calculated between the two populations separated by the river ("cross-river populations," Table 3). Fst is defined here as the cross-river genetic variance divided by total genetic variance for each population pair; it is bound between 0 and 1, reflecting no genetic differentiation and full differentiation, respectively. We ran a Mann–Whitney U test on the final Fst dataset to check if Fst values differed between the two types of genomic data (WGS and RAD-seq). Also, because geographic sampling distance among individuals varied across studies, we calculated the geographic sampling distance within each population to determine if higher within-population sampling distance affected cross-river Fst values and calculated a Mann–Whitney U test on those measurements (Supplementary Table S1).

Table 4 Organism dispersal table with estimated dispersal distance for species used in this study.

Species	Taxon	Dispersal (km)	Dispersal group/Rank		Reference
			G	Thr, 4	
Arizona elegans	Snake	5.0	H	3	Blouin-Demers and Weatherhead 2002[a]
Crotalus atrox	Snake	3.5	H	3	Landreth 1973
Crotalus scutulatus	Snake	2.0	L	2	[a]
Junco hyemalis oreganus	Bird	5.0	H	3	Liebgold et al. 2013[a]
Masticophis flagellum	Snake	1.0	L	1	Johnson et al. 2007
Peromyscus leucopus	Mouse	6.8	H	4	Maier 2002
Pituophis catenifer	Snake	0.7	L	1	Bishop et al. 2016
Plantago ovata	Herb	3.1	H	2	Iwanycki Ahlstrand et al. 2019; Meyers and Liston 2008; Vahabi et al. 2008[b]
Quercus ellipsoidalis	Tree	1.6	L	2	Axer et al. 2021; Crow 1988
Quercus rubra	Tree	1.6	L	2	Axer et al. 2021; Crow 1988
Rhinochelius lecontei	Snake	5.0	H	3	Blouin-Demers and Weatherhead 2002[a]

If a species did not have literature on the dispersal ability, the genus was used as an approximation. If not available, the taxonomic classification was used to group the organism. [a] Estimation made also using data from NatureServ Explorer (https://explorer.natureserve.org). [b] Estimated based on equation in Methods. Dispersal classifications are given for binary groups, G (H = high dispersal, L = low dispersal) and threshold ranking with four levels (Thr. 4) where 1 is lowest and 4 is highest. For full classifications see Supplementary Information.

Effective dispersal classification

To account for how species dispersal mediates the effects of a riverine barrier, we considered relative *effective* dispersal. Effective dispersal includes periodic long-range and animal-mediated dispersal because these impact genetic ancestry and relatedness. We estimated relative effective dispersal here in two ways. First, the genomic data were divided into two datasets, high dispersal and low dispersal ("high" and "low," respectively) based on estimates from literature and databases (Table 4); this was based on the rationale that these groups would be affected differently. Second, to determine if dispersal *itself* could be incorporated directly into the model, we included it explicitly as a ranked (ordinal) variable. This had the additional benefit of including all species in a single dataset. For this approach, we used different ranking systems to examine whether models were sensitive to variable specification. We ranked dispersal into three and four groups (dispersal, I3 and I4, respectively) and formed those groups using two different methods: an even number of members per group ("even" dataset), and a method where the range of dispersal values was divided by the number of desired ranks (3 or 4) to give the width of each dispersal bin, then species were sorted into those bins accordingly ("threshold" dataset). If a quantitative dispersal estimate could not be found for a species, dispersal was estimated based on other members of the genus or was estimated based on characteristics of its taxonomic group (for groupings see Supplementary Table S7).

One of our species, *Plantago ovata*, normally has limited seed dispersal, but is thought to periodically undergo animal-mediated long-distance transport. For this species, we therefore calculated a weighted dispersal average as 99/100 dispersal events occurring locally (0.1 km) and 1/100 events occurring long-distance (300 km), which gave a mean value of 3.1 km. These estimates are consistent with genetic work showing some genetic structure over medium-short distances while other studies find no population structure more than 100 s of kilometers (Vahabi et al. 2008; Shryock et al. 2021).

Estimating geographic sampling distance

The genomic data used in this study came from different sources and collecting strategies, and sampling distances varied. To account for and decouple the effect of geographic distance (isolation by distance) between cross-river populations from river-specific effects, we calculated the minimum Euclidean distance between individuals of the cross-river populations in each dataset using individuals' GPS locations. We chose minimum distance with the rationale that this would most closely reflect the opportunity these populations have to interact in the absence of a physical barrier.

River hydrology: Discharge and seasonality

We obtained river discharge data from the USGS National Water Information System (U.S. Geological Survey 2020) for gauges closest to where the genomic data were located based on visual inspection in Google Earth. For each USGS gauge, we batch downloaded daily discharge data for the three years between 1 January 2019 and 31 December 2021 using the dataRetrieval package (v2.7.11, Cicco et al. 2022) in R and recorded discharge in units of ft^3/second. This timeframe was chosen because it was the longest period that contained data for all discharge sites; it resulted in 1 to 22 discharge sites per river segment (median = 3; Table 5). From these data, we calculated the average daily discharge across discharge sites per river segment. The discharge sites for Stockton, CA (San Juaquin River) and Tucson, AZ (Santa Cruz River) were removed because they were in or near urban centers and/or were too engineered to obtain accurate river width and hydrology measurements.

Seasonality was calculated using daily discharge values averaged per river segment in two different ways: mean seasonality ratio (SR) and seasonality range over mean (ROM). SR was calculated by dividing the mean daily discharge of one hydrologic

Table 5 Data for each river and river segment used in this study.

Name	Average daily discharge ± SE (ft/s^3)	SR ± SE	Seasonality ROM	Width mean (km)	Width SD	No. discharge sites
Colorado- *elegans*	7040 ± 77	1.03 ± 0.03	0.90	0.53	0.50	4
Colorado- *ovata*	11,900 ± 79	1.49 ± 0.01	0.86	0.26	0.11	2
Croton	201 ± 5	0.47 ± 0.03	1.93	0.06	0.28	4
Deschetes	3270 ± 35	0.81 ± 0.02	0.52	0.20	0.11	3
Gila	88 ± 3	1.01 ± 0.06	2.29	0.88	0.60	8
Housatonic	1610 ± 29	0.64 ± 0.03	1.74	0.32	0.36	2
Kawishiwi	355 ± 9	0.85 ± 0.03	3.45	0.16	0.11	3
Kettle	833 ± 8	0.90 ± 0.08	3.44	0.87	0.25	1
Knife	81 ± 5	0.70 ± 0.10	4.43	0.06	0.05	1
Mississippi	13,300 ± 215	0.85 ± 0.03	2.27	0.79	0.91	4
Mojave	8 ± 2	0.04 ± 0.01	5.91	0.35	0.20	3
Muddy	43 ± 0	0.91 ± 0.01	0.27	0.59	0.35	2
Nemadji	423 ± 23	0.64 ± 0.07	3.38	0.36	0.08	1
Rio Grande- *atrox*	573 ± 8	1.02 ± 0.02	1.74	0.61	0.69	9
Rio Grande- *catenifer, scutulatus*	415 ± 11	3.98 ± 0.18	2.69	0.11	0.05	3
Rio Grande- *elegans, flagellum*	614 ± 8	0.92 ± 0.02	1.86	0.57	0.60	12
San Juaquin	940 ± 26	0.39 ± 0.02	1.50	0.52	0.43	8
San Pedro	26 ± 3	5.61 ± 0.62	6.44	0.16	0.21	4
Santa Cruz	30 ± 2	2.60 ± 0.32	3.25	0.20	0.12	6
St. Louis	1133 ± 42	0.59 ± 0.04	3.31	0.22	0.24	2
Pecos	69 ± 1	2.26 ± 0.05	2.55	0.42	0.52	22
Virgin	193 ± 5	0.29 ± 0.01	2.25	0.27	0.20	3
Wallkill	741 ± 17	0.38 ± 0.02	2.07	0.12	0.17	3

River segments are sections of a river paired to a specific genomic dataset; some genomic datasets spanned different portions of the same river, these are indicated by a suffix after the hyphen. Both measures of seasonality (ratio and range over mean, ROM) are reported, along with standard errors (SE).

season (1 July to 30 September) by another (1 January to 31 March) across discharge sites at each river segment then averaged over the 3 years (2019 to 2021). These dates were chosen to represent the monsoon season of the southwestern US where many studies were located, which leads to flashy (i.e. highly variable) discharge in this region (Sheppard et al. 2002). The timing of wet/dry seasons varies across the United States and was not further assessed in the study, but investigations of the effect of discharge variability in future studies is warranted.

For average ROM, the daily discharge per river segment was averaged by month; the range of mean monthly discharge values within the summer monsoon season was divided by the mean of monthly discharge values for that year. The per-year ROM was then averaged over the three sampled years (2019 to 2021). Because this measurement of discharge variability is standardized by average discharge, the values are more comparable across rivers of different sizes.

River shape: Width

River width was measured in kilometers at five locations at or near each discharge station and the mean, median, and SD of these values was calculated per river segment. Width calculations were made manually in Google Earth using the measurement tool and included visible floodplains delimited by visible depositional features, high escarpments, and/or riparian zones. Therefore, river width here measured the channel belt, encapsulating more than just the wetted river area (Supplementary Fig. S2). Without obvious features delimiting the floodplain, only the width of the actual river was used. We avoided urban areas where it was difficult to see channel features and where most rivers were leveed as evidenced by sections of anomalously straight river. Measurements near dams were avoided and instead moved a sufficient distance up- or downstream to avoid the associated areas of pooling or low flow. Finally, the river mouth where it meets the ocean was excluded as the geometry of this area generally differs from the rest of the channel.

Generalized linear and generalized linear mixed models

To determine what we could learn about river characteristics and population differentiation through traditional statistics we used a generalized linear model (GLM). The GLM was included as a positive control to test whether a traditional multivariate framework recovered the same relationships identified by the SEM models. To account for taxon-specific differences, we also ran a generalized linear mixed model (GLMM) with taxonomic group set as a random effect. For GLMs, in R the glm() function in the lme4 package (v1.1-31, Bates et al. 2015) was used. Fst was designated as the dependent variable while seasonality, mean discharge, and mean river width were independent variables; the same model was run

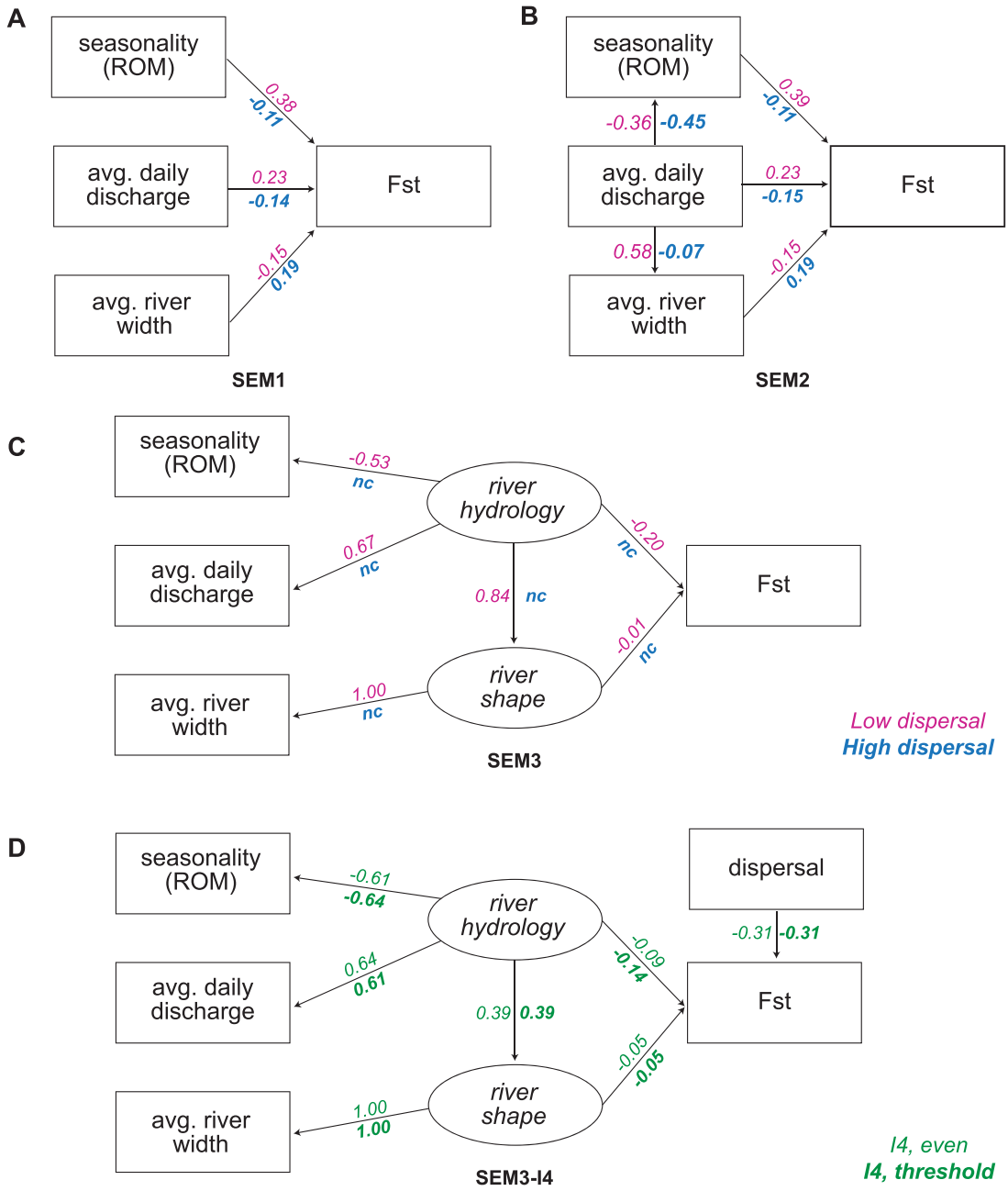


Fig. 2 Results of the structural equation models (SEM1–3) without geographic distance. Standardized path coefficients are shown for (A–C) the low dispersal (pink, values above/left of path) and high dispersal (blue, values below/right of path) datasets, and (D) dispersal specified using four even groups labeled in green (I4, even, values above/left of path) and four groups by threshold (I4, threshold, values below/right of path). Circles represent latent variables, rectangles represent manifest (measured) variables. NC stands for runs that did not converge. No individual path is signifiant.

separately for each method of characterizing seasonality (SR and ROM) and with all six dispersal datasets (low, high, I3-even, I3-threshold, I4-even, I4-threshold). The same approach was taken for the GLMMs using the glmer() function. We used a Gaussian link function in both sets of models based on exploration of the data distribution.

Constructing directed acyclic graphs

We built a set of DAGs (DAG 1–3) that articulate known relationships among river variables and their anticipated effect(s) on genomic differentiation (Table 2). The variables were selected because they were available across all systems, represent fundamental hydrological and physical properties of rivers, and are straightforward to interpret. DAGs assume there are no directed cycles or loops within the graph structure, and we do not have reason to believe this study violates that assumption. Each graph structure was chosen *a priori* for a specific theoretical or analytical purpose.

DAG1 is the simplest graph structure and articulates the three river variables with directed paths to Fst and no other relationships among them (i.e. no interdependence or internal river structure; Figs. 2a and 3a). This structure is equivalent to the GLM structure, providing a positive control by allowing

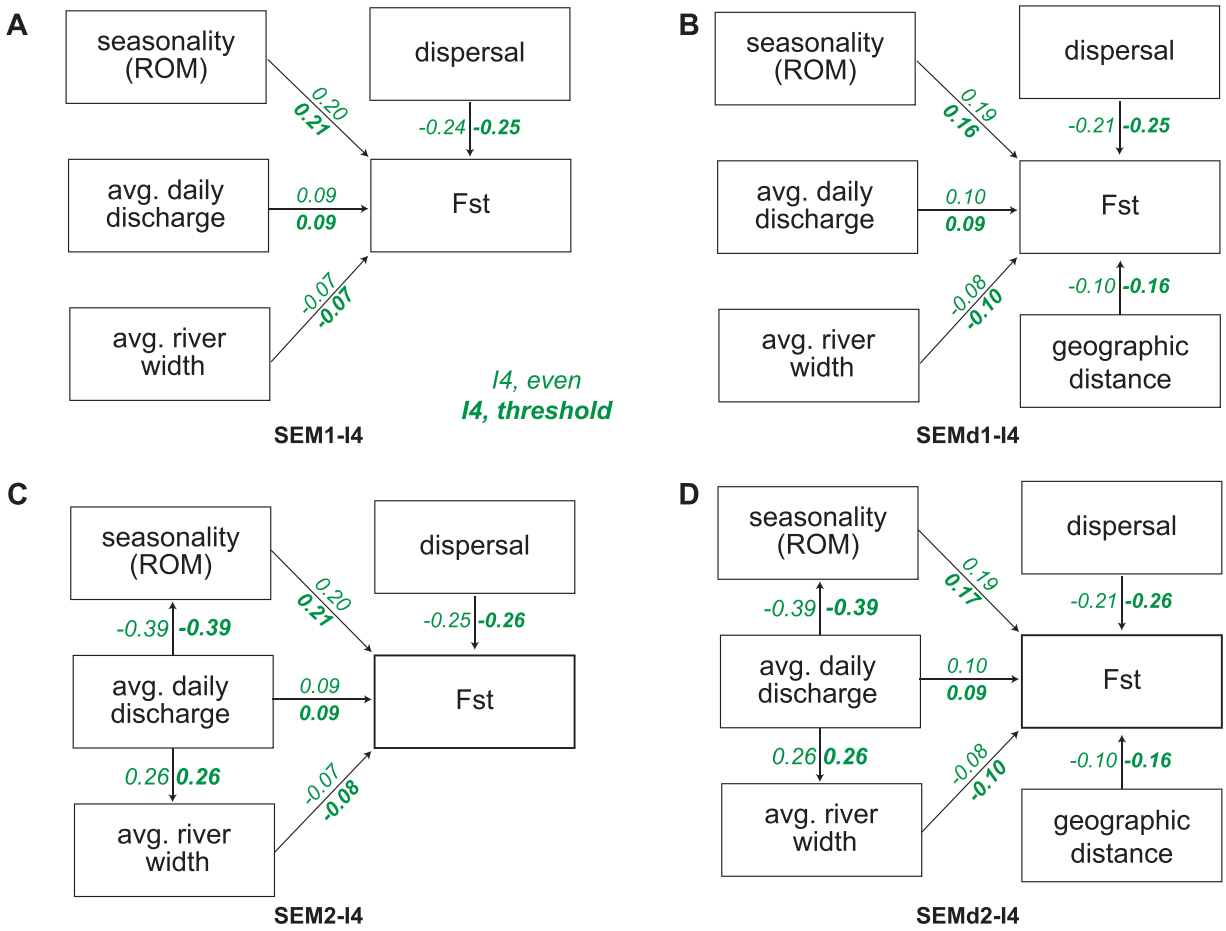


Fig. 3 Results of the structural equation models accounting for dispersal as an explicit parameter both without distance (a) SEM1-I, (c) SEM2-I and with distance (b)SEMd1-I, (d) SEMd2-I. Standardized path coefficients are shown for I4 datasets in which dispersal was ranked into four groups using an even clustering method (not bold, above/left of path) or threshold clustering method (bold, bottom/right of path). No individual path is significant.

a direct comparison to a traditional multivariate statistical approach.

DAG2 articulates the paths in DAG1 plus the known empirical relationship in which river width is proportional to river discharge (Leopold and Maddock 1953). Secondly, because our calculations of seasonality were calculated directly from average daily discharge, and because higher daily discharge associates with lower seasonal variability, there is an additional path between mean daily discharge and seasonality. Therefore, in addition to direct pathways, DAG2 has two indirect pathways to Fst through river width and seasonality that are known from first principles, published literature, and our calculations. If seasonality is derived from independent data in the future (i.e. climatological data instead of discharge data), it is possible this path would be better specified as bidirectional. Comparing the results from DAG1 and DAG2 structures allows us to assess if we learn anything about population differentiation by accounting for the internal structure of river systems.

DAG3 is most complex and includes latent variables, which are conceptual or difficult to measure variables. Latent variables are important for representing theoretical constructs that may be incompletely or imperfectly represented by a single variable but are nonetheless important to our understanding of a system or phenomenon (common examples are happiness or intelligence). In this case, DAG3 represents our conceptual model of river impacts on population structure, separating river effects into two fundamentally different types: hydrological and physical. The effect of river hydrology on river shape is specified in this model through a directed path; river shape is measured by the sole manifest (i.e. measured) variable of river width. Despite having a sole variable, we use this structure to illustrate that river shape is a *conceptual* variable that can be described by many aspects of river morphology. Future studies could incorporate additional indicators, like categorization of river type (alluvial or bedrock), sinuosity, depth, or channel complexity depending on the data available.

High and low dispersal datasets and ROM/SR were analyzed under these three DAG structures (DAGs1–3) with SEM analysis. In a second round of modeling, dispersal was included as a variable for DAGs1–3 using ROM only (ROM performed slightly better than SR, see *Results*). In a scenario where there is no riverine barrier effect, the expectation is that the path coefficients of directed paths leading to Fst would be low or zero. Using the same graph structure with different datasets allows the results of those paths to be directly compared.

Structural equation Modeling

All SEMs were run in R using lavaan (v0.6-11, Rosseel 2012) assuming linear relationships. Standardized and unstandardized

path coefficients, total effects, and global model fit indices (comparative fit index [CFI], root mean square error of approximation [RMSEA], standardized root-mean squared residual [SRMR]) were calculated using the sem(), summary(), and fitMeasures() functions. Graphs were generated with the semPaths() function and due to differences in scale among variables, we interpreted only standardized path coefficients. Support for specific paths within SEM2 and SEMd2 (best supported models) and R^2 values were calculated with inspect() and parameterEstimate(). Structural equation models were run for DAGs1–3: (1) without geographic distance or dispersal (hereafter, SEM1–3), (2) with geographic distance but without dispersal (hereafter, SEMd1–3), (3) without geographic distance but with dispersal (SEM1–3-I), (4) and with both geographic distance and dispersal (SEMd1–3-I). When dispersal was not explicitly modeled (SEM1–3 and SEMd1–3), models were run with high and low dispersal datasets separately.

Running high and low datasets separately through the same structure allows all intercepts and slopes among parent–child variables to vary. If an organism's dispersal mode makes it sensitive to different aspects of a river, the expectation is this will yield different path coefficients. If high dispersal reduces the impact of riverine barriers, we expect low model support as well as non-significant and small path estimates relative to low dispersers. Results from the dispersal-explicit datasets do not allow the slopes of parent–child variables to change, yielding the most generalizable model.

Model revision

Because DAGs are often built to articulate knowledge or theory of a system that is not yet fully understood, a key utility of SEM is the considered reciprocity between empirical model results and theory of that system. In fact, one often reaches new insights simply by building the DAGs. *Post-hoc* model revision is one opportunity for theoretical-empirical reciprocity in which new insights gained by the SEM process can be used to re-specify the models and run the data through them anew. That said, the spirit of SEM is to evaluate the fit of data to a model, not to develop the best-fitting model for a dataset. This is because the end-goal is a generalizable understanding of the *system* and any dataset is only one instantiation of that system (which may be biased or incomplete), and model revisions should be kept to a minimum. For all models described up to this point, we performed confirmatory SEM, allowing us to test our existing theory about riverine barriers. After reviewing the results of the 36 models, we moved into exploratory SEM by performing one round of model revision in which we *post-hoc* ran SEMd2 and SEMd2-I4-threshold a second time with an additional internal path from mean river width to geographic distance (SEMd2-alt, SEMd2-alt-I4-threshold; see *Discussion* for justification). In total, 38 model runs were completed (Supplementary Table S6).

As another post-hoc examination, we preliminarily evaluated whether the conflicting WIDTH → F_{ST} path across models (see *Results*) could be driven by narrower rivers inhabiting regions of higher elevation and topography. To do this we measured cross-section profiles of the rivers at discharge sites in this study using Google Earth and recorded the high and low points of those transects as a proxy for topographic relief. After averaging transects per river segment we performed a linear regression against river width.

Results

Genomic data curation and cross-river Fst

Our final dataset included genomic data from 26 population pairs of 11 species distributed across 20 different rivers; some papers produced multiple datasets, and some genomic datasets spanned different parts of the same river ("river segments"). The number of polymorphic loci per dataset ranged from 7,931 to 205,000 (Supplementary Table S11). Based on STRUCTURE analysis, *Anolis carolinensis* had two individuals that represented a separate population cluster on one side of the Savannah River. Once these two individuals were removed, the dataset did not meet minimum sample size (i.e. three individuals per population) and was excluded from further analysis. Eight individuals were removed from *P. ovata* dataset for the Mojave River. The best *K*-value for *Crotalus atrox* across the Gila River was 3, but there was no clear structure—all individuals had similar admixture (Supplementary Fig. S8)—so this dataset was retained. All other population pairs had a best *K* of either 1 or 2, suggesting we are not including non-river associated population structure in our curated dataset (for all results see Supplementary Fig. S8 and Table S12).

For the curated final dataset, Fst values ranged from 0.00 to 0.28 with a slight bias towards low values (Supplementary Fig. S3). Although Fst values from RAD-seq data were higher than WGS (0.09 vs. 0.07; Supplementary Fig. S4), there was no significant difference detected via two-tailed Mann Whitney U test ($P = 0.11$; $\alpha = 0.05$) after testing for data normality with a Shapiro–Wilk test ($P = 0.00$), so data from these studies were combined (Supplementary Fig. S4). This is consistent with prior results (Araya-Donoso et al. 2022). Approximately half the datasets had individuals sampled at only one location on each side of the river and half had individuals that were sampled across a small sampling distance. The latter studies had slightly lower cross-river Fst scores (0.06 vs. 0.09), which was just significant by a two-tailed Mann Whitney U test ($P = 0.04$, $\alpha = 0.05$). However, these different datasets were evenly distributed among high and low datasets, reducing its effect on model results. High dispersers and low dispersers had different Fst values as expected, but this was not significant (0.05 vs. 0.12; $P = 0.09$, $\alpha = 0.05$). We note that evaluating other complementary measures of population differentiation such as Da or Dxy would be worthwhile in future work. For bivariate relationships among all variables see Supplementary Fig. S7.

River hydrology and shape

Average daily discharge ranged across orders of magnitude (8 to 13,300 ft^3/s), suggesting our study captured a high degree of variation in river size and climate, though we had relatively fewer high-discharge rivers; this also accounts for the fact that discharge increases exponentially downstream. The Mojave River (in California) and San Pedro River (in Arizona) had lowest flow, while the Colorado River (in Arizona) and Mississippi River (in Minnesota) had highest flow. Measures of seasonality ranged from 0.04 to 5.61 for SR and 0.27 to 6.44 for ROM. The two metrics indicate similar patterns with Muddy River (Nevada) and Deschutes River (Oregon)

making up the low end of seasonality and the San Pedro River (Arizona) and Mojave River (California) marking the high end of seasonality. This ordering suggests these seasonality measures captured meaningful climate differences across the United States, for example southwest rivers experience summer monsoons and are expected to have higher discharge fluctuations than rivers in climates with more consistent precipitation, like the Pacific Northwest. For simplicity, in the main text we only report results for models using ROM because they are better normalized across river size (Supplementary Fig. S5). Mean river width varied from quite narrow for the Croton River (0.06 km) and Knife River (0.06 km) to wider for the Mississippi River (0.79 km) with a mean of 0.49 km across river segments.

Generalized linear models and mixed models

The coefficients of the GLMs showed very low effect size of river characteristics on Fst, with river width showing the strongest effect on both low and high dispersers (Supplementary Table S2). The regression coefficient for river width on Fst was negative for low dispersal datasets and positive for high dispersers; the discharge regressions were near zero (Supplementary Table S2). The GLMM showed no variance explained by taxonomic group, indicating taxonomic group may not be a good descriptor of barrier effects (Supplementary Table S3), however it is likely that this analysis was underpowered and could change with larger sample.

Structural equation modeling

The SEM1 (Fig. 2a) unstandardized path coefficients were identical to the GLMs (including signs), indicating the SEM analysis behaved as expected (Supplementary Table S2). Global model fit indices (CFI, RMSEA, and SRMR) met the threshold for good model fit, but this is an artifact of the model being just-identified (i.e. having zero degrees of freedom [DF]) and no *P*-value was calculated and fit indices are not reported (Table 6). The standardized path coefficients in SEM1 (unlike the unstandardized coefficients) showed seasonality was most explanatory for low dispersers, followed by discharge and then width, while high dispersers had similar magnitude of effects among predictors but were oppositely signed (Fig. 2a).

For SEM2, standardized path coefficients of direct paths to Fst were identical to those of SEM1, but this model included paths for internal river structure. Models that explicitly included dispersal (SEMd) estimated large, negative direct effects on Fst (−0.21 to −0.26) that reduced other path estimates (Fig. 3). When dispersal was modeled explicitly, the signs of paths were consistent among SEMs and GLMs. However, when analyses were split between high and low dispersal datasets, the signs of the coefficients varied between datasets. Thus, allowing path slopes and intercepts to change indicates that high and low dispersers are described by fundamentally different dynamics. The DISCHARGE → WIDTH → FST and DISCHARGE → FST paths could be affected by limited data because of the high dynamic range of discharge relative to the low sample size of the high and low datasets. However, this could also be interpreted as a habitat effect (see *Discussion*). All global model fit indices passed their respective thresholds for good model fit for high and low dispersal datasets including a non-significant *P*-value, but metrics were worse when dispersal was modeled explicitly (Table 6, Supplementary Table S4). This could be due to imprecise classification of species' dispersal abilities based on available literature, an underpowered dataset that would benefit from more paired population replicates, or be due to constraining the slopes of parent–child variables across all types of dispersers.

Using an endogenous latent variable structure, SEM3 presented inconsistent results with SEM2: SHAPE → FST was negligible for low dispersers and HYDROLOGY → FST was medium magnitude and negative (Fig. 2C). The SEM3-I4 results had medium weight on dispersal and were consistent across even and threshold datasets (Fig. 2D). The runs for high dispersal did not converge, which could reflect that they are less impacted by river systems. Non-convergence can also arise from small sample size or DF, model complexity, or high multicollinearity.

Overall, SEM3 model fits were generally poor. While model complexity or identification issues could play a role, SEM3 and SEM2 had the same degrees of freedom and both were identified, although latent variable structure is often considered more complex. There were three indications of poorer fit. First, analogous paths of SEMs 1 and 2 were consistent while SEM3 differed. Second, two of the runs for SEM3 did not converge (Table 6, Supplementary Table S4). Third, global fit indices for SEM3 were mixed overall but worse than SEM2, producing poor CFI and RMSEA values and a marginal SRMR value. These results could reflect the limited data available in this study with the wide variance of discharge and river width variables relative to sample size. It could also be due to the lack of a directed edge from discharge to seasonality in SEM3. We do not necessarily think this reflects limited utility of latent variables in this context. The contiguous US has highly variable seasonality, terrain, and types of rivers—we suggest that in other settings these SEMs may be sufficiently powered, particularly if covering a smaller range of river types.

SEMs with geographic distance

When explicitly modeled, the DISTANCE → FST standardized path had a very high estimate (−0.49, −0.23 for SEMd2), suggesting the isolation by distance effect is being accounted for, with a greater effect on low dispersers as expected. Surprisingly, including distance in the models greatly increased the WIDTH → FST standardized path from −0.15 to −0.52 for low dispersers and increased the DISCHARGE → FST standardized path (0.23 to 0.42) while having negligible effect on high dispersers (Fig. 2 vs Fig. 4; Supplementary Table S5). Modeling dispersal explicitly (Fig. 3D) negated this effect.

To address the negative DISTANCE → FST relationship for both high and low dispersers, we respecified SEMd2 to include an internal path from river width to geographic distance (SEMd2-alt, Fig. 5) to make this relationship explicit. The path estimate for low dispersers was negative while for high dispersers it was positive but near zero. For low dispersers, the variance partitioning (−0.57, −0.53, −0.55) may reflect multicollinearity. Alternatively, this re-specification offers a new biologically meaningful interpretation: (1) the *total* effect of river width via physical distance for low dispersers ($-0.53 \times -0.55 = 0.29$) and high dispersers ($0.05 \times -0.23 = -0.01$) captures the expected positive relationship for low dispersers and the difference in magnitude expected between them due to isolation by distance; (2) so, the opposing

Table 6 Summary of model results and global fit indices for SEM models.

Model	DF, S/B	Dataset	Chi-square *P*-value	CFI	RMSEA	SRMR
SEM1	*0/3*	*Low dispersal*	–	–	–	–
		High dispersal	–	–	–	–
SEM2[a]	1/6	Low dispersal	**0.33**	**1.00**	**0.00**	**0.08**
		High dispersal	**0.75**	**1.00**	**0.00**	**0.02**
SEM3	1/6	Low dispersal	**0.20**	0.78	0.24	0.09
		High dispersal	Did not converge			
SEMd1	*0/4*	*Low dispersal*	–	–	–	–
		High dispersal	–	–	–	–
SEMd2[b]	3/9	Low dispersal	**0.08**	0.56	0.34	0.21
		High dispersal	**0.75**	**1.00**	**0.00**	**0.07**
SEMd3	4/10	Low dispersal	**0.07**	0.39	0.32	0.24
		High dispersal	Did not converge			
SEMd2-alt	3/10	Low dispersal	**0.37**	**0.98**	**0.06**	**0.12**
		High dispersal	**0.67**	**1.00**	**0.00**	0.09
SEM1-I4	*0/4*	*I4, even*	–	–	–	–
		I4, threshold	–	–	–	–
SEMd1-I4	*0/5*	*I4, even*	–	–	–	–
		I4, threshold	–	–	–	–
SEM2-I4	3/9	I4, even	0.05	0.47	0.25	0.13
		I4, threshold	**0.13**	0.62	0.19	0.11
SEMd2-I4	5/12	I4, even	**0.13**	0.50	0.16	0.12
		I4, threshold[b]	**0.18**	0.61	0.14	0.11
SEM3-I4	4/10	I4, even	**0.08**	0.48	0.20	0.14
		I4, threshold	**0.19**	0.67	0.14	0.12
SEMd3-I4	7/14	I4, even	**0.25**	0.61	0.11	0.14
		I4, threshold	**0.32**	0.78	0.08	0.12
SEMd2-alt-I4	6/14	I4, even	0.04	0.26	0.21	0.16
		I4, threshold	**0.22**	0.59	0.12	0.12

Reported are CFI, RMSEA, SRMR and DF for both the specified user model and the baseline model (S/B). A value less than 0.08 is considered good for SRMR and RMSEA; a value over 0.90 is considered good for CLI (Xia and Yang 2019). For the Chi-square goodness of fit test, a *P*-value *greater* than 0.05 is considered an acceptable model. SEM1 and SEMd1 are saturated (have zero DF) and therefore fit indices are not reported ("-"). Dataset reports how dispersal was modeled: either as separate runs (low, high dispersal) or as an ordinal variable in the SEM with three (-I3) or four (-I4) ranks where rankings were determined by an even or threshold clustering method. Overall, SEM2 is the best-fit model ([a]), with SEMd2 and SEMd2-I4 also good fitting models ([b]); some runs did not converge. Values meeting the threshold for good model fit are bolded.

direct effect sign of river width → Fst for low (−0.57) and high (0.20) dispersers may reflect specific river effects of topography and habitat unsuitability, respectively. To better understand if the conflicting Width → FST path could be due to narrower rivers associating with higher topographic relief at elevation, we regressed our proxy for topographic relief against river width, which showed a weak but negative relationship (Supplementary Table S6). The Distance → Fst and Width → Distance individual paths in the SEM2d-alt model were statistically significant (P = 0.044 and 0.039 respectively; Fig. 5, Supplementary Table S8), but these interpretations require further testing.

Technical effects of dispersal specification

Different binning methods for dispersal had little effect on overall model results and internal paths. When included, the direct path from dispersal to Fst was always strongly negative, suggesting insensitivity to variable specification. Global fit indices and path estimates for models with explicit dispersal were worse overall than those where high and low dispersers were run separately. This could be a power issue, but we instead believe it to be biologically meaningful. That the model results for high and low dispersers strongly differed indicates the model structure to capture river barrier effects of each fundamentally differs. Explicitly including dispersal in the model does not reconcile this fundamental difference because it does not allow the slopes among parent–child variables to change as a function of dispersal, it only varies the intercepts. This model constraint implies that all species are affected in the same way by each variable, just to different strengths. Based on the results herein we now believe this not to be true. Qualitatively, dispersal-included models gave path values intermediate to the values of low and high disperser datasets, hinting at broad consistency among models.

Choosing the best SEM

Most specific paths were not significant, but Width → Fst and Distance → Fst for low dispersers in SEMd2 showed significant

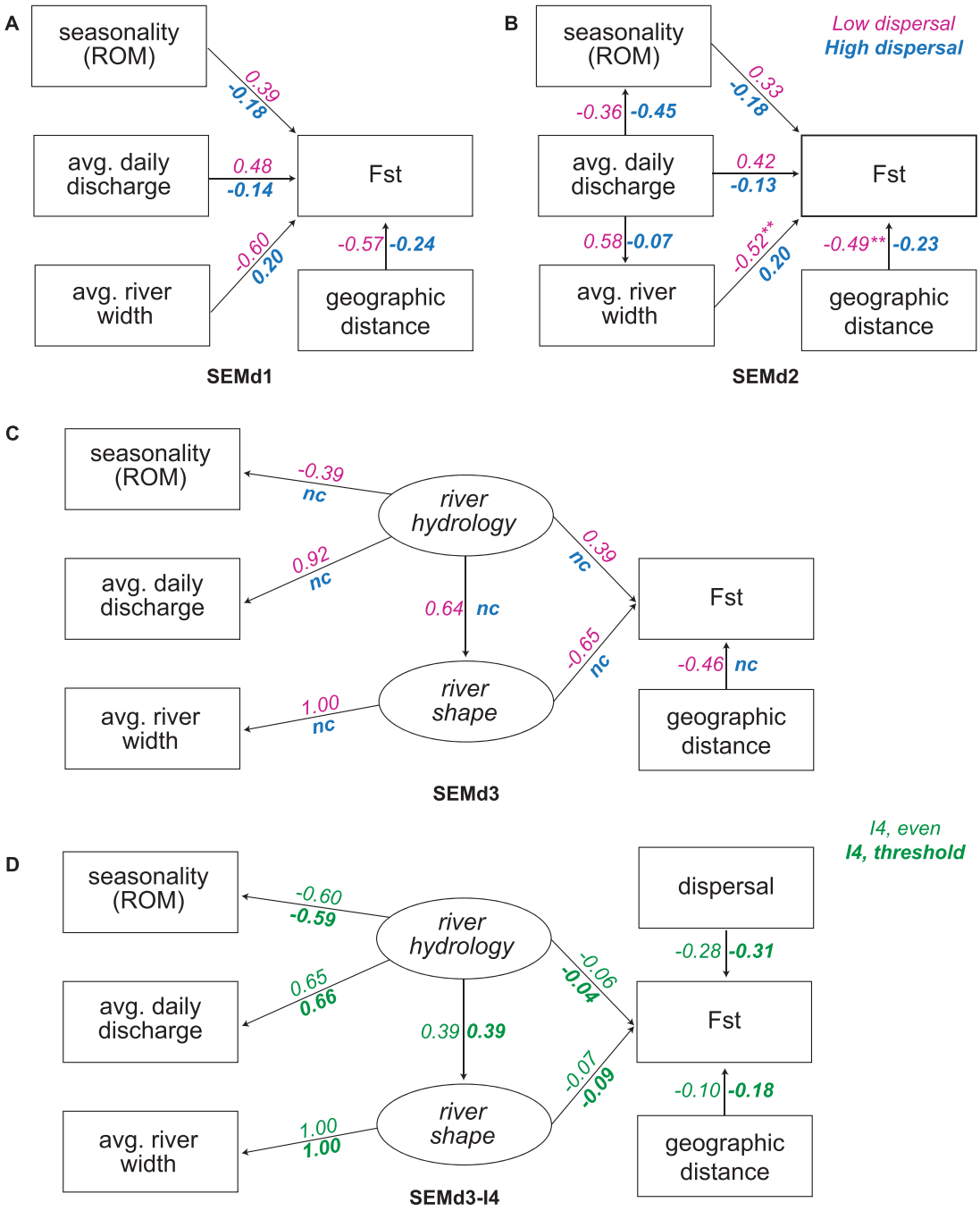


Fig. 4 Results of the structural equation models (SEMd1–3) accounting for geographic distance within the structure. Standardized path coefficients are shown for (A–C) the low dispersal (pink, values above/left of path) and high dispersal (blue, values below/right of path) datasets, and (D) dispersal specified using four even groups (I4, even, values above/left of path) and four groups by threshold (I4, threshold, values below/right of path). **Individual paths whose 95% CIs do not overlap zero and are significant (Supplementary Table S8).

P values and 95% CIs that did not span zero (Supplementary Table S8). SEM2 (and to a lesser degree SEMd2) had excellent global fit indices, both reflect the relationships we know to be important, and both are biologically interpretable. Surprisingly, SEMd2 predicted 52.1% of Fst variance for low dispersers (Supplementary Table S9)—much more than SEM2 (R^2 = 14.7%)—suggesting that this model describes riverine barrier systems well for the most affected taxa and that both isolation by distance and barrier effects are effectively captured. However, we note that this number could be slightly inflated by having removed admixed individuals from the datasets. As expected, the model was less explanatory for high dispersers (R^2 = 5.9% to 12.9%), reflecting the decoupling of barrier effects under higher gene flow. Despite lower model fit (Table 6) the SEMd2-I4-threshold model may best represent the causal structure of this system because it integrates all taxa in one framework. That said, we learned the most by comparing the SEM2 model results between high and low dispersers. SEMd2 can be viewed as a specialized case of the SEMd2-I4-threshold model, optimized for low-dispersal dynamics. Hereafter we focus our interpretations on SEM2 and SEMd2 for their biological interpretability when compared between high and low dispersers.

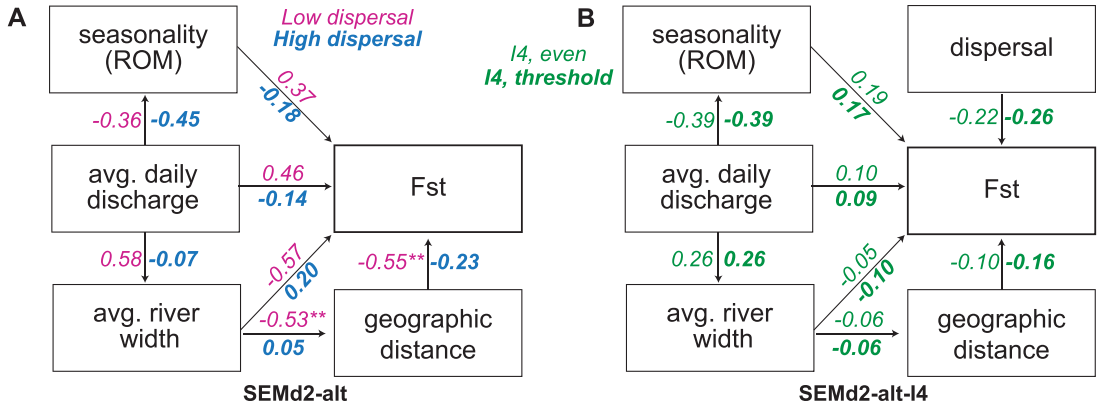


Fig. 5 Results of the structural equation model for SEMd2-alt which is the model from SEMd2 with an additional path to account for the relationship between river width and geographic distance. Models were run with (A) high and low dispersal separately, and (B) with dispersal explicitly modeled into four ranked dispersal groups (I4) using even and threshold clustering methods (above/below path, respectively). Standardized path coefficients are shown.**Individual paths whose 95% CIs do not overlap zero and are significant (Supplementary Table S8).

Within SEM2, SEMd2, and SEMd2-alt, the *total effect* of discharge on Fst (direct and indirect paths) was low or zero for low dispersers 0.00, 0.00, 0.17) and high dispersers (−0.11, −0.06, −0.07), but oppositely signed. Its indirect paths cancel out its medium to large direct effect on Fst in most cases. In contrast, the direct effect of width on Fst, which was −0.15, −0.52, −0.57 in low dispersers and 0.19, 0.20, 0.20 in high dispersers remained high when considering *total* effects: −0.15, −0.52, −0.28 for low dispersers and 0.19, 0.20 and −0.20 for high dispersers (Supplementary Table S10). Considering total effects shows that compared with discharge, river width generally has a higher effect on Fst than would be indicated by non-structured multivariate analysis (i.e. GLMs). It also demonstrates that when geographic distance is accounted for, it changes the variance structure in a way so that the effects of river width and discharge are easier to detect (Fig. 2B vs 4B).

Discussion

This study sought to evaluate the longstanding riverine barrier hypothesis by capturing elements of river complexity in DAGs using population genomic data of 26 plant and animal populations spanning 23 river regions. GLMs indicate low overall predictive power of seasonality, river discharge, and river width on genetic differentiation of low and high dispersers (Supplementary Table S2). SEM1 mirrored the direct-only path structure of the GLM and yielded identical results, but standardized coefficients showed that seasonality or discharge were most influential on Fst (Fig. 2). SEM2 and SEMd2 we interpret to be more complete models relative to GLM/SEM1 (Fig. 2), reflected by very good global fit indices (Table 6) and the fact that SEMd2 predicted 52% of Fst variance for low dispersers, which is the set of taxa we expect to be most affected by river barriers. The better fit of SEM2 models demonstrates that incorporating the internal relationships among river components resulted in a more nuanced understanding of how rivers influence species evolution. Explicitly modeling dispersal performed worse than models using only low dispersers (Fig. 3), but results indicate the SEMd2-I4-threshold model is probably the best overall generalization of the riverine barrier effect.

Using traditional unstructured statistics (SEM1), we would have concluded that seasonality and discharge were most important for population structuring. GLM-equivalent model (SEM1) would suggest that low dispersers are most affected by seasonality. Accounting for system structure through internal paths (SEM2 models) revealed the direct effect of discharge on population differentiation is canceled out by its indirect effects on Fst through other river components (Supplementary Table S10). Its negation leaves width as the main influencer of Fst among high and low dispersers when geographic distance was not included. Modeling distance allowed partitioning of that variance, increasing the effect of other riverine variables, particularly for low dispersers.

Modeling the internal structure of rivers

We interpret SEM2 and SEMd2 to be the best-fitting structures because they account for relationships known to exist among the river variables and therefore allow apportioning of variance without overly complicating the graph structure. The path coefficients given in SEM2 support known features of river systems for all datasets. Average daily discharge had a negative (inverse) effect on discharge seasonality, conforming to our expectation that smaller rivers (i.e. those with lower mean discharge) tend to be flashier (i.e. have more variable flow) than larger, higher-discharge rivers (Baker et al. 2004).

SEM2/SEMd2 confirm the known positive relationship between discharge and river width in all models except for high dispersers where that value was consistently negative but near zero; this probably reflects the decoupling of high-dispersal species dynamics from river effects, as we expect the high disperser dataset to not reflect river dynamics as well as others. As flow accumulates downstream, channel width, depth, and flow velocity increase to accommodate the higher water discharge (Leopold and Maddock 1953). The relationship is nonlinear (Leopold and Maddock 1953), and therefore may not be best represented by the partial linear regression performed in our SEM, although this path could be specified differently in future work. Larger datasets would help resolve what, if any of these conflicts are due to insufficient power versus real biological differences.

Effect of river width on Fst

Both GLMs and SEM1 showed that genetic differentiation negatively associated with river width for low dispersers but not high dispersers (Fig. 2A, Supplementary Table S2). This remained true

in the SEM2 models (Fig. 2B). The negative relationship for low dispersers is counterintuitive, stating that the narrower the river, the higher the genetic differentiation. The consistency across SEM and GLM methods (Supplementary Table S2) suggests it could be a feature of the dataset, and indeed the bivariate relationship between Fst and river width appears to be very weakly negative (Supplementary Fig. S7). However, it could also reflect a real component of the system—one where high and low dispersers are affected fundamentally differently by river width. High dispersers may be challenged by wider rivers, wider floodplains, and associated changes in vegetation. This would also be expected to be true of low dispersers, but the counterintuitive negative WIDTH → FST path for low dispersers may be explainable by the topographic setting of rivers: at higher elevation, rivers are closer to their headwaters and associated with higher topographic relief. Toward headwaters, less flow has accumulated, and channels are typically narrower—as exhibited by the DISCHARGE → WIDTH relationships shown in the models. Topographic relief could thus pose an additional, inversely related barrier to cross-river movement, driving differentiation; this would yield a negative WIDTH → FST relationship among low dispersers, while maintaining the positive WIDTH → FST relationship observed for high dispersers, who may be less affected by topography and more affected by floodplain habitat or changes in vegetation.

These conflicting WIDTH → FST results align with previous findings in an Amazon frog species where higher genetic divergence was associated with the Upper Napo River, characterized by higher topographic relief, than the wider Lower Napo River (Funk et al. 2007), meanwhile the opposite pattern was recently observed for two bird species (Bennett et al. 2025; Mikkelsen et al. 2025). The negative WIDTH → FST for low dispersers may therefore reflect topographic effects, while the positive relationship for high dispersers reflects the challenge with traveling longer distances over unsuitable habitat. We suggest explicitly incorporating trait-based effects (e.g. Paz et al. 2015; Papadopoulou and Knowles 2016) would be a worthwhile addition to resolve this effect. It would also offer an opportunity to integrate other phylogeographic advances with those in this study.

Another explanation for negative WIDTH → FST path could lie in facultative dispersal (Ruokolainen et al. 2019). Rivers are not static features of landscapes, and some rivers shift more than others (Musher 2025); those with a more dynamic history can promote more cross-channel gene flow than those that have remained stationary (Ribas et al. 2022, 2025). Alluvial rivers, which have channels formed by sediments, tend to be more dynamic than bedrock rivers whose position is controlled by underlying geologic features (Schumm 1985). Alluvial rivers experience processes such as meander cut-offs and avulsions, both of which frequently reconnect terrestrial populations and lead to reticulation. In our study, many of the wide rivers fall into the more dynamic alluvial category (e.g. Mississippi) while narrower bedrock rivers may have had a more fixed position over time (e.g. Colorado, Rio Grande), which would explain a negative WIDTH → FST relationship. In a comparative study of Amazonian birds, Johnson et al. (2023) found greater landscape stability in forest uplands led to higher genetic structure than in more dynamic lowland environments. The contiguous US encompasses a wide range of elevations and topographies that might permit detection of topographic effects and differences among river types more than in the Amazonian lowland systems where much riverine barrier work has been done. Adding river classification to future models may better resolve the WIDTH → FST relationship. Fortunately, SEM makes such additions tractable.

Do rivers affect high dispersers?

We used high dispersers as a comparison group, expecting they would be less affected by river attributes than low dispersers. Supporting this, path estimates were almost always smaller magnitude for high dispersers and SEMd2 explained Fst variance much less for high than low dispersers (13% vs. 52%; Supplementary Table S9). The path coefficients between high and low dispersers often varied, suggesting that population differentiation in these groups are characterized by different phenomena that are best represented with fundamentally different parent-child relationships in the analysis (see *Results*). Modeling dispersal explicitly further showed this as it produced results intermediate to high and low dispersers, which can be viewed here as end-members.

Additional factors may have inflated river-associated effects as the herb psyllium (*P. ovata*) was represented in the high disperser dataset and in the southwestern (highly seasonal) rivers. There is evidence that this species is locally adapting, evidenced by outlier loci that may be under selection (Shryock et al. 2021) and is capable of selfing (Sharma et al. 1993). Both could increase Fst unrelated to effects of a river and could have biased the SEM results. Its placement in the high dispersal group, if anything, should inflate the importance of river paths, reducing the true differences between high and low dispersers. Additional biological attributes that could affect results are sex-biased dispersal, incorrectly estimated dispersal abilities, and historical demographic effects such as bottlenecks, all of which could be investigated with additional data.

Limitations and caveats of sampling and method

Results of this study could be biased in two ways by the data sources used. First, the geographical sampling area of each study varied. We found this had a small but marginally significant effect on Fst with greater geographic sampling slightly suppressing cross-river Fst values. However, this should result in underestimating river effects. This could be true for *Mastocophis flagellum* whose sampling covered larger ranges than other studies where individuals were collected at a single site (e.g. *P. ovata*; Supplementary Table S1).

Second, dispersal was difficult to measure for some organisms, and estimates conflicted at times particularly between published literature and publicly available databases. For example, we estimated dispersal of oaks (*Quercus* spp.) as 1.6 km based on Axer et al. (2021) who performed a regeneration density study on oaks in Germany. They found a maximum estimated dispersal of 2.29 km, but most dispersal is low to zero beyond ∼1.6 km (see Fig. 5 in Axer et al. 2021), while an older study estimates dispersal of 4 to 5 km (Crow 1988). The herb *P. ovata* is generally expected to have low dispersal (Ellner and Shmida 1981). However, congeners are animal-dispersed including by birds, and their seeds (like those of *P. ovata*) form mucilage when wet, suggesting animal mediated dispersal is relevant for *P. ovata* (Cavers et al. 1980;

Phan et al. 2020; Kreitschitz et al. 2021; Abe et al. 2022). This would increase its effective dispersal ability. This species is also part of a group where long-range dispersal is well documented (Iwanycki Ahlstrand et al. 2019); its colonization of North America from the Mediterranean is thought to have been natural (200 to 650 kya, Meyers and Liston 2008) and genetic evidence shows this species can have low population structure over hundreds of kilometers, indicating effective dispersal (Vahabi et al. 2008; Shryock et al. 2021). In summary, dispersal is not a discrete value but a distribution whose shape varies by taxon, and sometimes by setting; its estimation by a single number was a necessary simplification for our analyses.

To our knowledge, there are no missing confounders in our models, which are variables that affect both predictor and outcome variables and can lead to spurious conclusions. However, there are likely to be additional abiotic or biotic factors affecting Fst of individual studies, which might impact the statistical power of these analyses. For example, species-specific differences in historical effective population size are likely to affect genetic diversity and its geographical structuring. However, this is to a degree an inherent trait of the organism, and we do not have a conceptually meaningful way to specify this in our models. It is also possible that dispersal ability and geographic distance may covary in some applications of this framework. If so, variance may be partitioned across multiple paths, potentially affecting path coefficients, standard errors, and causal interpretation. In our study, geographic distance reflects the Euclidean distance between sampling locations, whereas dispersal reflects a species trait, and we therefore treated them as separate predictors.

Finally, our results revealed very high intrinsic variance in components of this system (river discharge and width, dispersal ability), which may leave our dataset underpowered in some instances, and is a cautionary note for all studies of river barrier effects. Improving statistical power in the future could be achieved by: (1) deliberately targeting or limiting environmental and taxonomic heterogeneity in sampling, (2) increasing population replicates per species across different segments of the same river, and (3) expanding taxonomic coverage within each river system.

Use of DAGs in geogenomic systems

Many of the physiographic features thought to be important for structuring populations (rivers, mountains, land-sea connectivity) are multifaceted, leading to complex and context-dependent expectations (Ribas et al. 2022). Representing individual components within a graph allows their articulation and quantification, assessment of their direct and indirect relationships, and their comparison across contexts (Dolby 2021; Schrodt et al. 2025). More than that, the earth processes that form these features are inherently interconnected and these interdependencies are often understood at the level needed to construct such graphs. These structures offer opportunities to collaborate across fields, taxonomic dimensions, and between empiricists and theorists to ensure proper model specification and alignment.

The results here offer a more nuanced understanding of how rivers may structure populations but does not resolve the riverine barrier hypothesis. It did, however, restructure our thinking. For example, the negative path from river width to Fst suggests modeling regional topography and historical channel movement are important (Ribas et al. 2025), and the difference in sign suggests river width may affect population structure of low and high dispersers in fundamentally different ways. It also revealed that while river discharge has a strong direct impact on Fst for low dispersing organisms, it exerts other impacts on the system that mitigate that effect, leaving instead a large impact of river width through both direct and total effects (perhaps through topography). Overall, low dispersers are affected much more than high dispersers, and the two types of taxa are explained by fundamentally different phenomena. Our global fit indices and R^2 values for Fst show these models explain low dispersing population structure surprisingly well. The relative low support for individual paths highlights the broad dynamic range of the variables of river-organism systems, explaining why conflicting results among individual studies of the riverine barrier hypothesis are, in retrospect, wholly expected. SEM gives an approach to learn from those intricacies.

The DAGs depicting the same causal structure can be applied in different studies. The SEMs used here could be readily implemented to rivers across China or to the large rivers in South America that originally inspired the hypothesis. The latter may be especially valuable due to the number of population genomic datasets and studies available. These models could be rerun with new data to determine which (if any) results were artifacts of the dataset. Adopting the same graph structure allows for aggregation of results beyond any individual study to reveal the conditions of river-mediated isolation, and probably other physiographic systems at the boundary of earth processes and biological evolution.

In short, we find that the graph-based approaches reveal attributes of the interaction between rivers and population evolution beyond what can be learned from non-structured techniques. This result marks a material advance in our ability to articulate and ascribe putatively causal relationships within Earth-life systems (Dolby 2021; Igea and Tanentzap 2021; Dong 2022; Vernham et al. 2023; Schrodt et al. 2025) and integrate geological and genetic datasets more broadly—a fundamental need within convergence science (Dawson et al. 2022; Dolby et al. 2022; Ribas et al. 2022).

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Author contributions

Garett L. Maag (Formal analysis, Investigation, Methodology, Writing—original draft), Austin R. Biddy (Data curation, Formal analysis, Methodology, Validation, Writing—original draft), Maya F. Stokes (Investigation, Writing—review & editing), and Greer A. Dolby (Conceptualization, Formal analysis, Project administration, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing)

Supplementary material

Supplementary material is available at *Journal of Heredity* online.

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Data availability

KML of individuals used and R scripts are available at https://doi.org/10.5061/dryad.s1rn8pkq5. All sequence data used in this study was publicly available and accession numbers are available in the supplemental information.

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