

# A river runs through it: Causal graphs capture rivers' complex control on the genetic structure of populations

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## Abstract

Earth's physiographic features shape the genetic evolution of organisms. Understanding the conditions under which such features act as barriers to gene flow requires quantifying and articulating the features of both the barrier and the organism(s). Many such physiographic features, however, have known interdependencies that are not expressed through common multivariate statistics. Here, we evaluate the use of directed acyclic (causal) graphs and structural equation modeling (SEM) to articulate and test these relationships. We chose the longstanding and contested Riverine Barrier Hypothesis as a test-case using 28 river-spanning population genomic datasets of plants and animals associated with 25 rivers across the contiguous United States; data were paired with seasonality, river width, and river discharge data for those rivers. SEMs revealed insights that could not be captured by traditional non-structured multivariate statistics. Discharge had the greatest direct effect on low-dispersing species. However, discharge has negative, indirect effects on other river features making its total effect on population differentiation negligible. River width was important for low dispersers, but surprisingly, narrower rivers were associated with higher  $F_{st}$ —this may be due to the association of higher topography with narrower (e.g., headland) parts of rivers. Or, wide lowland rivers may be more dynamic and facilitate dispersal more than highland rivers. Therefore, topography or landscape history and not wetted river area may determine barrier efficacy. This proof of concept shows the utility of causal graphs and SEM at articulating and testing complex relationships between Earth's physiographic features and the organisms that evolve with them.

## INTRODUCTION

The riverine barrier hypothesis Wallace originally articulated in 1854 proposes 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 is expected to occur. 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. Since then, numerous studies have tested this hypothesis across taxa, climatic and geographic settings, and types of rivers using mark-recapture, species surveys, and (most commonly) molecular data to assess population structure across river channels. Yet, results have been highly variable and at times directly conflicting (Table 1), making the riverine barrier hypothesis a topic of great debate within comparative phylogeography and biogeography. 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 only 13 out of 28 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 also been mixed in primates (Boubli et al., 2015; Jalil et al., 2008; Link et al., 2015) and amphibians (Fonseca et al., 2021; Fouquet et al., 2015; Gehring et al., 2012; Godinho & Da Silva, 2018), but rivers are often found to *not* be a barrier among small mammals (Colombi et al., 2010; Klee et al., 2004; Roratto et al., 2015).

Intuition suggests that whether rivers restrict dispersal would depend on the mode of dispersal and the traits or ecology of an organism. High-dispersing species of trees and birds would be impacted to a lesser degree or not at all. Yet that is not always the case, at least for a species of Amazonian tree (Nazareno et al., 2019a) and an African bird (as well as its parasitic lice; Voelker et al., 2013). In China, Zhang et al. found that the Yangtze River restricts seed dispersal in a large shrub but not its pollen dispersal, leading to male biased cross-river gene flow (Zhang et al., 2007). This example makes sense because pollen can be carried farther distances on the wind than many seeds. As shown by these examples, the relationship between organismal characteristics and river-associated divergence is much more varied than intuition would expect, suggesting aspects of these systems have not yet been sufficiently captured.

While a wide breadth of organismal characteristics have been considered, comparably little attention has been paid to the characteristics of the rivers themselves (but see Dolby et al., 2019; Jackson & Austin, 2013; Ruokolainen et al., 2019). Larger river channels should serve as stronger barriers than smaller branches or tributaries, which was a pattern observed in a shrub species in Amazonia (Nazareno et al., 2017). Some have predicted that wider river mouths are stronger barriers than narrower headwaters, and this has received mixed support (Figueiredo-Vázquez et al., 2021; Godinho & Da Silva, 2018; Patton et al., 1994; Peres et al., 1996). However, it could also be argued that although high elevation portions of rivers are narrower, they may have greater cross-valley topographic relief and these more dramatic elevation changes could restrict dispersal more than the flatter, wider low elevation parts of the same rivers. There are many aspects of rivers that vary, including but not limited to their slope, width, water discharge, and flow characteristics; these variables are often interrelated (Leopold et al., 1964; Leopold & Maddock, Thomas, 1953; Park, 1977) and each may have its own role to play in barrier effects. 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 rivers without fully accounting for the diversity or complexity of both.

Resolving the above question requires three analytical advances. First, aspects of the proposed river barrier must be sufficiently quantified and in a standardized way to allow direct comparison among them both within and across rivers. Second, the interdependency of the riverine variables must be appropriately considered. Third, the statistical framework must integrate primary riverine and population divergence data in a generalizable way such the results of one system can be directly compared to another, avoiding some of the noise from study to study. Ideally, the framework would capture the complexities of both the river and organism and in so doing allow us to ask the more nuanced question of, “*what are the conditions* under which rivers isolate or structure populations?” as opposed to asking, “*do* rivers structure populations?”. Where, the results of the latter are bound to be mixed.

In this study we aimed to satisfy these requirements and evaluate the riverine barrier hypothesis by analyzing genomic data from published literature for 28 population pairs of plant and animal species traversing 25 river systems across the contiguous United States (Figure 1). We obtained river discharge, discharge seasonality, and river width measurements for locations where population genomic data was available across a river channel. We first used traditional multivariate statistical techniques to assess which river characteristic(s), if any, explain genetic differentiation among organisms, and compared these to results from a graph-based statistical approach, structural equation modeling (SEM). SEM is based on directed acyclic graphs (DAGs) that capture known direct and indirect relationships (i.e., pathways; Table 2) among variables (Figure S1). In this case, known empirical relationships among riverine attributes were captured and their individual and interactive controls on genomic differentiation ( $F_{st}$ ) were assessed.

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 multivariate analyses. This result marks a material advancement in our ability to articulate and ascribe putatively causal relationships within Earth-life systems (Dolby, 2021; Dong, 2022; Igea & Tanentzap, 2021; Vernham et al., 2023) and integrate geological and genetic datasets more broadly—a fundamental need within convergence science (Dawson et al., 2022; Dolby et al., 2022).

## MATERIALS & METHODS

### *Curation of genomic data*

We mined published literature for population genomic datasets that met several criteria, the data: i) were publicly accessible, ii) provided GPS coordinates for sampled individuals, iii) contained at least three individuals per sampled population, and iv) had populations separated by a river channel (see Table S1 for data sources). Only terrestrial species were chosen for analyses as aquatic, semiaquatic, or amphibious species likely disperse within river systems and could complicate results. Datasets used either whole genome sequencing (WGS) or Restriction-Site Associated DNA sequencing (RADseq). Importantly, the original studies did not necessarily generate data to study riverine barriers, and so the datasets may reflect additional features or evolutionary patterns that we did not address and may therefore increase variance in our models. For each paper, we recorded the genus and species, organism group, accession numbers, GPS coordinates, and type of genomic data (WGS or RADseq). The final dataset included birds, grasses, lizards, rodents, snakes, and trees (Table 3).

### *Calculation of $F_{st}$*

We calculated  $F_{st}$  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 (links to data are in Table S1). 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 & Grünwald, 2017) in R Studio (v.1.3.1073, R Core Team, 2021) and individuals were grouped into three populations, two groups that represent individuals closest to the river on either side, and a third group consisting of more geographically distant individuals (this third group was not used). The `genind` file was converted to a `heirfstat` file using the `heirfstat` package (v0.5-11, Goudet, 2005) and a pairwise  $F_{st}$  value was calculated between the two river-associated populations (Table 3). We ran a T-Test on the final  $F_{st}$  dataset to check if  $F_{st}$  values were biased between the two types of genomic data (WGS and RADseq).

### *Dispersal Classification*

Based on the expectation that high and low dispersing species would be affected differently by a riverine barrier, the genomic data were divided into two datasets: high dispersal and low dispersal based on estimates from available literature and databases (Table 4). High dispersal organisms are the species in our data set that have the potential to travel farther due to dispersal modes. 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.

### *River hydrology: Discharge and seasonality*

We obtained water discharge data from the USGS National Water Information System (U.S. Geological Survey, 2020) for discharge stations closest to where the genomic data were located based on visual inspection in Google Earth. For those specific discharge sites we batch downloaded daily discharge data between January 01, 2019 and December 31, 2021 using the `dataRetrieval` package (v2.7.11, Cicco et al., 2022) in R and recorded discharge in units of 1000 ft<sup>3</sup>/second. This timeframe was chosen because it was the longest period that contained data for all discharge sites and this resulted in between 1 to 22 discharge sites per river segment (Table 5). With these data we calculated the average daily discharge across discharge site per river segment. The discharge sites for Stockton, CA (San Joaquin River); Tucson, AZ (Santa Cruz River); Columbus, GA (Chattahoochee River); and 280 Atlanta, GA (Chattahoochee River) were removed because they were in or near urban centers and/or were too engineered to obtain accurate and ‘natural’ river width and hydrology measurements.

Seasonality was calculated over daily discharge values averaged per river segment in two different ways: mean Seasonality Ratio (SR) and seasonality Range over Mean (ROM). The Seasonality Ratio (SR) is calculated by comparing the mean daily discharge of one hydrologic season (July 1<sup>st</sup> to September 30<sup>th</sup>) by another (January 1<sup>st</sup> to March 31<sup>st</sup>) across discharge sites at each river segment and then this ratio was averaged over the three years (2019–2021). These dates were chosen to represent the monsoon season of the southwestern

US which leads to flashy (i.e. highly variable) discharge in this region (Sheppard et al., 2002). The timing of wet/dry seasons may vary across the US and was not further assessed in the study.

For average ROM, the daily discharge 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 (2019–2021). Because this measurement of discharge variability is standardized by average discharge, the values are more comparable across rivers of different sizes.

#### *River shape: Width*

The river width was measured in kilometers at five locations within or near each discharge station and the mean, median, and standard deviation of these values was calculated per river segment. Width calculations were made in Google Earth using the measurement tool and these measurements included visible floodplains delimited by alluvial fan deposits, high escarpments, and/or riparian zones. Therefore, river width here measured the ‘channel belt’, encapsulating more than just the wetted river area. Without obvious features delimiting the floodplain, only the width of the actual river was used (Figure S2). We avoided urban areas where it was difficult to see channel features and where most rivers were leveed as evidenced from anomalously straight portions of the river. Measurements near dams were avoided and instead taken a sufficient distance upstream or downstream to avoid the pooling or low-flow areas associated with dams. Finally, the river mouth where it meets the ocean was also excluded as the geometry of this area is often unlike the rest of the channel. It is worth noting that we made a second dataset using a global river database (GRWL, Allen & Pavelsky, 2018); although it is comprehensive, it did not contain many smaller rivers in this study and so we did not use this dataset in further analyses.

#### *Estimating geographic distance*

The genomic data used in this study came from different sources and so collecting strategies likely differed. To account for the effect of geographic distance between sampled populations, for each genomic dataset we calculated the minimum Euclidean distance between individuals of the two cross-river populations using individuals’ GPS locations. We chose minimum distance as opposed to mean or maximum distance with the rationale that this would most closely reflect the opportunity these populations have to interact in the absence of a physical barrier.

#### *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) as well as a generalized linear mixed model (GLMM) to determine the effect of taxonomic group. For GLMs, 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 and the same model was run on the four datasets: seasonality (SR and ROM) and dispersal groups (high and low). The same approach was taken for the GLMMs except the `glmer()` function was used instead and the taxonomic group was set as a random effect. We used a Gaussian link function in both sets of models based on exploration of the data distribution.

#### *Constructing Directed Acyclic Graphs (DAGs)*

We built a set of DAGs (DAG 1–3) that articulate known relationships among the river variables in this study and their anticipated effect on genomic differentiation (Table 2), measured as `Fst`. 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 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; Figure 2a). This structure is equivalent to the GLM structure, allowing a direct comparison to a more traditional multivariate

statistical approach that could reasonably be used to understand such relationships, and therefore serves as a positive control.

DAG2 articulates the paths in DAG1 plus the known empirical relationship in which river width is proportional to river discharge (Leopold & Maddock, 1953). Secondly, because our calculations of seasonality contained averaged daily discharge, there is an additional path between mean daily discharge and seasonality. Therefore, in addition to direct pathways, DAG2 has two indirect pathways to  $F_{st}$  through river width and seasonality that are known from first principles or published literature. Comparing the results from DAG1 and DAG2 structures allows us to assess if we learn anything by accounting for the internal structure of river systems.

DAG3 is the most complex and includes latent variables, which are conceptual or difficult to measure variables. Latent variables are particularly important for representing theoretical constructs that may be incompletely represented by a single variable but are nonetheless important to our understanding of the system (common examples are happiness or intelligence). In this case, DAG3 shows that the river variables can be conceptualized into two types: hydrology and shape. 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.

All four datasets were analyzed under these three DAG structures (DAGs1–3) with structural equation modeling analysis. Under a scenario where there is no riverine barrier effect, the expectation is that the path coefficients of directed paths leading to  $F_{st}$  would be low or zero.

### *Structural Equation Modeling (SEM)*

All SEMs were run in R using lavaan (v0.6-11, Rosseel, 2012). Standardized and unstandardized path coefficients, total effects, and global model fit indices (CFI, RMSEA, SRMR) were calculated using the `sem()`, `summary()`, and `fitMeasures()` functions. Graphs were generated with the `semPaths()` function and due to the differences in scale among variables, we interpreted mainly standardized path coefficients. Structural equation models were run for DAGs1–3 without geographic distance (hereafter, SEM1–3) and with geographic distance (hereafter, SEMd1–3). Each model was run for high and low dispersal datasets as well as ROM and ratio seasonality measurements (four datasets each), resulting in a total of 24 model runs.

Because DAGs are often built to articulate the 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 models themselves. *Post-hoc* model revision is one such opportunity for theory-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 as the goal is a generalizable understanding of the system and any given dataset is only one instantiation of that system (and which may be biased or incomplete). Thus, model revisions should be kept to a minimum. With this in mind, after reviewing the results of the 24 models, we performed one round of model revision in which we *post-hoc* ran SEMd2 (the best-fitting model) a second time with an additional path from mean river width to geographic distance (SEMd2-alt; see discussion for justification).

## **RESULTS**

### *Genomic data curation and cross-river $F_{st}$*

Our final dataset included 28 genomic population pairs from 12 species distributed across 25 different rivers; some papers produced multiple datasets and some genomic datasets spanned different parts of the same river (referred to as “river segments” in methods).  $F_{st}$  values ranged from 0.00 to 0.39 with a skew towards low  $F_{st}$  values (Figure S3). There was no significant difference in  $F_{st}$  detected via the two-tailed T Test between WGS and RADseq data ( $p = 0.31$ ) after removing one value (*P. ovata*,  $F_{st} = 0.33$ ) that was a significant outlier (Dixon’s test run on RADseq data,  $p = 0.004$ ), so these were combined in analyses (Figure S4). This

is in line with another study that did similar analyses and also did not find significant differences in Fst between RADseq and WGS datasets (Araya-Donoso et al., 2022).

### *River hydrology and shape*

Average daily discharge ranged across several orders of magnitude (8–13,300 ft<sup>3</sup>/s), suggesting our study captured a high degree of variation in river size and climate. 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 the highest. Measures of seasonality ranged from 0.04–5.61 for seasonality ratio (SR) and 0.26–6.44 for range over mean (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 climate differences across the US, for example rivers of the southwest experience summer monsoons and are expected to have higher discharge variability than rivers in climates with more consistent precipitation like the Pacific Northwest. For simplicity, in the main text we only report results for the models using the ROM seasonality measurement because they are better normalized across river size and the variance of ROM was wider, suggesting perhaps it may be more discriminatory (Figure S5). Mean river width varied from quite narrow for the Croton River (0.06 km, NY) and Knife River (0.06 km, Minnesota) to very wide for the Savannah (2.72 km) and Chattahoochee (1.53 km) rivers (both in Georgia) with a mean of 0.52 km across all river segments.

### *Generalized linear models and mixed models*

The GLM showed very low explanatory power of river characteristics on Fst, with river width showing the largest of the three (-0.032) for low dispersers and seasonality highest (0.068) for high dispersers (Table S2). The regression coefficient for river width on Fst was negative for both high and low dispersers, which is counter to the expectation (Table 2) and the seasonality regression was positive while the expectation was it would be negative. The generalized linear mixed model showed no variance explained by taxonomic group. This may indicate that species responses to rivers were idiosyncratic, but it is likely that this analysis was underpowered and it is unlikely that would hold in a larger dataset (Table S3).

### *SEMs*

The SEM1 (Figure 2a) for low and high dispersal showed unstandardized path coefficients identical to the GLMs (Table S2), indicating that the SEM analysis behaved as expected. Global model fit indices (CFI-comparative fit index, RMSEA-root mean square error of approximation, and SRMR-standardized root-mean squared residual) met the threshold for good model fit, but this is an artifact of the model being saturated (i.e. having zero degrees of freedom). Tellingly, the Chi-square p-value for model fit was significant (p-value = 0), indicating the model and data are statistically significantly different from each other. This is reasonable since we know SEM1 excluded known relationships among river variables (Table 2).

Accounting for internal river channel structure, standardized path coefficients for SEM2 showed discharge had the largest direct impact on Fst for low dispersers and seasonality had the highest coefficient for high dispersers (Figure 2b), as was observed in SEM1. The signs of paths were consistent between high and low dispersal datasets which was expected. However, as observed in the GLMs, river width had a negative relationship with Fst, which was unexpected. All global model fit indices passed their respective thresholds for good model fit for SEM2 (Tables 6, S4). Additionally, the p-value of the model was not significant, which means that model and data are not statistically different from one another, also indicating good model fit.

Accounting for endogenous latent variable structure, SEM3 presented consistent results with SEM2 except that for high dispersers, the path from hydrology to Fst had a negative sign and the path coefficient from hydrology to shape shrank to near zero. While the degrees of freedom were the same for SEM3 and SEM2, the latent variable structure is broadly considered a more complex model, and it may be likely that the datasets were too small to adequately estimate these relationships. There are three signs of this. First, paths of SEMs 1 and 2 are consistent and SEM3 differs. Second, the run for SEM3 using seasonality ratio

instead of ROM for seasonality did not converge (Table S4), suggesting inadequate statistical power. Third, global fit indices for SEM3 were good overall but worse than SEM2, producing borderline SRMR values (0.09 and 0.10 for low and high dispersal, respectively) and 0.25 for RMSEA (good model fit should be  $< 0.8$ ). Finally, SEM3 does not include the direct path from discharge to seasonality and this may be a model misspecification. These results are likely more a reflection of the limited data available in this study, and not a true assessment of the utility of latent variables in this context.

Intriguingly, including geographic distance in the models had a large effect on paths among low dispersers but almost no effect on paths for high dispersers (Figure 2 vs Figure 3; Table S5), which makes biological sense. By adding geographic distance, the median percent change in the absolute value of direct paths to Fst between SEM1–3 and SEMd1–3 was 31% and 3% for low and high dispersing datasets, respectively (Table S5). By accounting for variance contributed to Fst by geographic distance, the effect of other variables became weaker in low dispersers with little change for high dispersers. The difference was consistent between models 1 and 2 with a percent reduction of 43% for discharge, 31% for ROM seasonality, and 17% for river width. As mentioned above, SEM3 may be a good model but is probably overidentified relative to the data provided. Indeed, SEMd3 had more dimensions of freedom and performed less well than SEM3 (Table 6), further suggesting more data would be needed to evaluate complex models.

### *Choosing the best SEM*

SEM2 was the best fitting model for both datasets. SEM2d was equally well fitting for the low dispersers, but not the high dispersers. This finding is biologically realistic as the increased model complexity of adding the path (distance - Fst) yields three additional degrees of freedom and would therefore result in poorer fit indices for high dispersers because, for them, that path is non-informative, so a simpler model is justified. So while SEM2d is not supported for high dispersers, it may in fact be the best model for low dispersers. With that understanding, we favor SEM2 and SEM2d and primarily focus our interpretations on these models.

Within SEM2, SEM2d, and SEM2d-alt, the *total effect* of discharge on Fst (direct and indirect paths) was near zero for low dispersers (-0.02, 0.14, 0.04) and high dispersing datasets (-0.04, -0.04, -0.07), respectively. This is because its negative indirect paths cancel out its positive direct pathway. This is in contrast to the direct effect of width on Fst, which was -0.37, -0.48, -0.24 for low dispersers and -0.1 in all SEM2 models for high dispersers. Considering the total effects shows that compared to discharge, river width has a much higher effect on Fst than would be indicated by non-graph-based multivariate analysis (i.e., GLMs).

## **DISCUSSION**

This study sought to evaluate the longstanding riverine barrier hypothesis by capturing elements of river complexity in directed acyclic graphs along with population genomic data of 28 plant and animal populations across 25 rivers. Generalized linear models indicate low overall predictive power of seasonality, river discharge, and river width on genetic differentiation of low and high dispersers, with similar coefficients for the three variables (Table S2). SEM1 mirrored the direct-only pathway structure of the GLM and was a poor fitting model based on chi square goodness of fit tests compared to SEM2 (see Results; Table 6). This result demonstrates how collapsing the internal relationships known to exist among the river variables results in a model that is unable to appropriately partition variance among the components of river systems.

The best fitting models (SEM2, SEM2d) reveal that when system structure is properly accounted for, the positive direct effect of discharge on population differentiation is canceled out by its negative, indirect effects on other river components. When considering direct effects only, discharge did have the greatest effect on Fst for low dispersers, and seasonality had the greatest effect for high dispersers (Figures 2b, 3b). But when considering total effects, the barrier effect of discharge is canceled out by its other indirect effects on the system, leaving river width and seasonality roughly equal influencers on Fst of low dispersers (SEM2: -0.37, 0.43, respectively), and seasonality as the single greatest control on Fst for high dispersers, which are affected little by discharge or river width as expected.

The signs of the effect of width on Fst and geographic distance on Fst were both negative, which is strongly

counterintuitive. However, this was consistent in both the GLMs and across SEMs, suggesting it is either an artifact of the dataset, or there are important components of this system not yet understood. Accounting for geographic distance between populations had a large effect on low dispersers and little effect on high dispersers, which makes intuitive sense even though the sign of this path is also opposite the expectation (Table 2; Araya-Donoso et al., 2022). This indicates the graph structure is able to disentangle the effect of geographic distance from the river barrier effect.

### *Modeling rivers as systems*

SEM2 and SEM2d were the best-fitting models, which we interpret to be because they account for relationships that are known to exist among the river variables without overly complicating the graph structure relative to the data supplied. SEM1 collapses internal pathways and redistributes that variance over direct pathways, and in doing so underestimates the importance of river width. The path coefficients given in SEM2 support known features of river systems. For example, average daily discharge has a negative (inverse) effect on discharge seasonality, which conforms 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). Another way to consider this is rivers that have high average discharge necessarily drain larger catchments, larger catchments capture more climate heterogeneity and are therefore less affected by local to regional weather patterns.

SEM2/SEM2d confirm the known positive relationship between discharge and river width, where segments of rivers with more water discharge are wider. As flow accumulates downstream, channel width, depth, and flow velocity increase to accommodate the higher water discharge (Leopold & Maddock, 1953). The magnitude of this pathway differed between high and low dispersers, which we interpret is likely due to limited sample size. The relationship between discharge and width is nonlinear (Leopold & Maddock, 1953), and therefore may be poorly represented by the partial linear regression performed in SEM and may also require more data to sufficiently capture the different parts of that relationship.

### *River width, distance, and Fst*

The GLM and SEMs revealed a negative relationship between river width and Fst (Figures 2, 3), which is counterintuitive and indicates that the wider the river, the lower the genetic differentiation associated with that river. It was expected that magnitude of effect of width on low dispersers would be higher than on high dispersers, which is observed. However, the negative sign of this relationship is either an artifact of the dataset or model, or it is a real aspect of the relationship. If real, it shows we mis-conceptualized our predictions (Table 2) and through the process of SEM have indeed learned something new. Additional data and analysis would reveal whether the sign of the relationship persists and therefore resolve if it is a data problem, a model problem, or an accurate reflection of the system.

If the negative path is true to the system, it could be due to the topographic setting of rivers: at higher elevation, rivers are closer to their headwaters and often associated with higher topographic relief. Topographic relief could pose an additional barrier to movement and in many cases it could be a primary driver of differentiation; this would yield a negative width-Fst relationship. Indeed, this result is consistent with prior results in an Amazon frog species where higher divergence was associated with the Upper Napo River, which hosts higher topographic relief, than the wider Lower Napo River (Funk et al., 2007). In a cursory attempt to assess this further, we took cross-section profiles of the rivers in this study at discharge sites using Google Earth, and recorded the high and low points of those transects as a proxy for topographic relief. After averaging discharge transects per river segment, a linear regression against river width showed a weak but negative relationship (Figure S6). The negative relationship between width and Fst can therefore be potentially accounted for by local effects of topography. However, more targeted sampling and modeling work is needed to evaluate these relationships in detail.

Another possible explanation for the negative relationship between width and Fst could lie in the history of the riverine barrier. Rivers are not static features of landscapes, and some rivers shift more than others. Rivers with a more dynamic history may promote more gene flow across the channel than those that have



remained stationary. Generally, alluvial rivers, which have channels formed by sediments, tend to be more dynamic than bedrock rivers whose position may be controlled by underlying geologic features (Schumm, 1985). Alluvial rivers experience processes such as meander cut-offs and avulsions, both of which may serve to frequently re-connect populations of terrestrial organisms. In our study, many of the wide rivers may fall into the more dynamic alluvial category (e.g., Savannah, Chattahoochee) while narrower bedrock rivers may have had a more fixed position over time (e.g., Colorado, Rio Grande). This comparison offers another possible explanation for the unexpected negative relationship between  $F_{st}$  and channel width observed in our dataset. Indeed, in comparative study of Amazonian birds, (Johnson et al., 2023) found that greater landscape stability in forest uplands led to higher genetic structure than in more dynamic lowland environments. This is another fruitful hypothesis generated from these SEM results that can be evaluated with future work.

Interestingly, geographic distance and  $F_{st}$  also had a negative relationship, counter to our expectation, however the coefficient for high dispersers was nearly zero (as expected). After reviewing results from the 24 models, we respecified SEM2d to include an internal path from river width to geographic distance (SEM2d-alt, Figure 4) to determine if the negative relationships was an effect of the river width to  $F_{st}$  path being negative. As expected for low dispersers, this pathway was positive and strong and so it is possible the negative effect of distance on  $F_{st}$  can be attributed to the same source as the negative relationship of river width to  $F_{st}$ . It is interesting, however, that for high dispersers all of these relationships are negligible, as expected.

#### *Do rivers affect high dispersers?*

This approach would be strengthened with better dispersal data for the species, or by targeting new data collection for organisms with different dispersal levels. We used the high dispersers as a negative control as they should not be affected by the river attributes to the same degree as low dispersers. Results supported this expectation, and discharge having a mid-to-low impact. For high dispersers, seasonality was by far the greatest control on  $F_{st}$ , with higher measures of seasonality associating with stronger population differentiation. We interpret this to be a broad climate signal that is difficult to interpret in more detail at this stage. It could also be an artifact of the dataset as the grass psyllium (*Plantago ovata*) was represented in many of the southwestern (highly seasonal) rivers. There is evidence that this species is locally adapting as indicated by outlier loci that may be under selection (Shryock et al., 2021) and is capable of selfing (Sharma et al., 1993). Both of these processes would increase  $F_{st}$  for reasons unrelated to a riverine barrier, and because of the frequency of the species in the greater dataset could bias our SEM results.

#### *Use of DAGs in genomics and biogeography*

Many of the physiographic features thought to be important to structuring or isolating populations (rivers, mountains, land-sea connectivity) are multifaceted, meaning their characteristics and their effect on biology can be explained in several ways. Representing individual facets within a graph structure allows their articulation and quantification, assessment of their direct and indirect relationships, and their comparison (Dolby, 2021). More than that, the earth processes that form and shape these features are fundamentally interrelated, and often these interdependencies are relatively well understood, at least at the level needed to construct such graphs. Doing so may motivate and indeed require substantive collaboration across the fields.

The results here offer a nuanced understanding of how rivers may structure populations, but by no means does it resolve the riverine barrier hypothesis. It did, however, restructure our thinking of these systems in a way that motivates new experiments. For example, the negative path from river width to  $F_{st}$  suggests that explicitly modeling regional topography, landscape history, and river width together may be important to resolving this hypothesis. It also revealed that while river discharge has a strong direct impact on  $F_{st}$  for low dispersing organisms, it exerts other impacts on the system that mitigate that effect.

The mixed evidence regarding the riverine barrier hypothesis to date may be a sampling effect where two multifaceted features, rivers and species, are compared without adequately quantifying the attributes of both. Perhaps the largest strength DAGs offer is the ability to apply the same causal structure to different

datasets and in doing so greatly reduce study-to-study variance, or at a minimum illuminate its possible sources. For example, the analyses done here within the contiguous US could be readily applied across rivers in China or to the large river systems in South America that were the original inspiration for this hypothesis. We could additionally collect more data for the rivers studied here and rerun the models to determine which relationships remain and which were artifacts of the data. Adopting the same graph structure allows for the aggregation of results beyond any individual study to better understand the conditions of river-mediated isolation, and probably many other physiographic systems at the boundary of earth processes and biological evolution.

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## DATA ACCESSIBILITY

All datasets and R scripts are available at <https://dataverse.harvard.edu/privateurl.xhtml?token=6461ccf6-d14c-421f-8c8a-b82e9959241a>. All sequence data used in this study was publicly available and accession numbers are available in the supplemental information.

## AUTHOR CONTRIBUTIONS

GM performed the research, analyzed the data and co-drafted the manuscript; MFS interpreted results and edited the manuscript; GAD conceived of, designed, supervised the research, provided resources, interpreted results and drafted the manuscript.

## TABLES & FIGURES

**Table 1** Summary of results from literature where the riverine barrier hypothesis was tested, including 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.

Reference	Taxon	Location	N	Result
Boubli et al., 2015	primates	Brazil	10	
Cáceres, 2007	small mammal	Brazil	4	
Colombi et al., 2010	Small mammal	Brazil	1	
Godinho & Da Silva, 2018	frogs	Amazonia	577	
Figueiredo-Vázquez et al., 2021	salamanders	Iberian Peninsula	1	
Fonseca et al., 2021	frogs	Brazil	1	
Fouquet et al., 2015	frogs	Guiana shield	28	
De Fraga & 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	

**Table 2** Description and justification of pathways used to build the directed acyclic graphs that were tested (DAG1–3; Figures 2–4).

#	Pathway	Expected relationship	Obs.	Justification of path
1	Discharge - seasonality	Negative	Yes	Higher discharge associated with larger catchment

#	Pathway	Expected relationship	Obs.	Justification of path
2	Discharge - river width	Positive	Yes	Higher water discharge associated with wider c
3	Discharge - Fst	Positive	Yes	Higher flow would be harder for organisms to s
4	River width - Fst	Positive	No	Wider rivers would be harder for an organism
5	Seasonality - Fst	Negative	No	High seasonality implies periods of low flow wh
6	River width - geographic distance	Mixed	Yes	Wider rivers have banks farther apart, therefor
7	Geographic distance - Fst	Positive	no	Isolation by distance principle, it is easier to d

**Table 3. Information for the 28 genomic datasets** used in this study, including associated references, sample sizes (N), associated river barrier, and data type (GBS – genotype by sequencing, RADseq – Restriction Associated DNA). Fst refers to the pairwise Fst calculated in this study for cross-river populations.

Group	Genus	Species	River	N	Fst	Data Type	Reference
Snake	<i>Rhinocelivus</i>	<i>lecontei</i>	Pecos	8	0.10	WGS	(Boubli et al., 2015)
Snake	<i>Arizona</i>	<i>elegans</i>	Colorado	8	0.09	WGS	(Myers et al., 2019)
Snake	<i>Masticophis</i>	<i>flagellum</i>	Rio Grande	8	0.03	WGS	(Myers et al., 2019)
Snake	<i>Masticophis</i>	<i>flagellum</i>	San Pedro	8	0.40	WGS	(Myers et al., 2019)
Snake	<i>Crotalus</i>	<i>scutulatus</i>	Rio Grande	8	0.04	WGS	(Myers et al., 2019)
Snake	<i>Pituophis</i>	<i>catenifer</i>	Rio Grande	8	0.07	WGS	(Myers et al., 2019)
Snake	<i>Pituophis</i>	<i>catenifer</i>	San Pedro	8	0.00	WGS	(Myers et al., 2019)
Snake	<i>Crotalus</i>	<i>atrox</i>	Gila	8	0.05	WGS	(Myers et al., 2019)
Snake	<i>Crotalus</i>	<i>atrox</i>	San Pedro	8	0.04	WGS	(Myers et al., 2019)
Snake	<i>Crotalus</i>	<i>atrox</i>	Rio Grande	8	0.02	WGS	(Myers et al., 2019)
Snake	<i>Crotalus</i>	<i>atrox</i>	Santa Cruz	8	0.02	WGS	(Myers et al., 2019)
Lizard	<i>Anolis</i>	<i>carolinensis</i>	Savannah	8	0.04	RAD-seq	(Manthey et al., 2016)
Lizard	<i>Anolis</i>	<i>carolinensis</i>	Chattahoochee	8	0.10	RAD-seq	(Manthey et al., 2016)
Rodent	<i>Peromyscus</i>	<i>leucopus</i>	Housatonic	15	0.12	WGS	(Munshi-South et al., 2016)
Rodent	<i>Peromyscus</i>	<i>leucopus</i>	Walkkill	17	0.02	WGS	(Munshi-South et al., 2016)
Rodent	<i>Peromyscus</i>	<i>leucopus</i>	Croton	15	0.06	WGS	(Munshi-South et al., 2016)
Grass	<i>Plantago</i>	<i>ovata</i>	Mojave	24	0.33	RAD-seq	(Shryock et al., 2021)
Grass	<i>Plantago</i>	<i>ovata</i>	Colorado	23	0.10	RAD-seq	(Shryock et al., 2021)
Grass	<i>Plantago</i>	<i>ovata</i>	Muddy	24	0.09	RAD-seq	(Shryock et al., 2021)
Grass	<i>Plantago</i>	<i>ovata</i>	Virgin	24	0.05	RAD-seq	(Shryock et al., 2021)
Tree	<i>Quercus</i>	<i>rubra</i>	Kettle	25	0.05	RAD-seq	(Quijano et al., 2020)
Tree	<i>Quercus</i>	<i>ellipsoidalis</i>	Mississippi	24	0.13	RAD-seq	(Quijano et al., 2020)
Tree	<i>Quercus</i>	<i>rubra</i>	St. Louis	28	0.08	RAD-seq	(Quijano et al., 2020)
Tree	<i>Quercus</i>	<i>rubra</i>	Knife	24	0.07	RAD-seq	(Quijano et al., 2020)
Tree	<i>Quercus</i>	<i>rubra</i>	Nemadji	26	0.13	RAD-seq	(Quijano et al., 2020)
Tree	<i>Quercus</i>	<i>rubra</i>	Kawaishiwi	24	0.28	RAD-seq	(Quijano et al., 2020)
Bird	<i>Junco</i>	<i>h. oreganus</i>	Deschutes	6	0.00	WGS	(Friis et al., 2018)
Bird	<i>Junco</i>	<i>h. oreganus</i>	San Joaquin	16	0.01	WGS	(Friis et al., 2018)

**Table 4. Organism dispersal table** with estimated dispersal distance for species used in this study. If a species did not have literature on the dispersal ability, the genus was used as an approximation. If no similar genus existed in the data of a species, the taxonomic classification was used to group the organism. +Estimation made also using data from NatureServ Explorer (<https://explorer.natureserve.org>).



Species	Taxon	Dispersal (km)	Reference
<i>Anolis carolinensis</i>	Lizard	0.06	(Quijano et al., 2020)
<i>Arizona elegans</i>	Snake	~1–10	(Blouin-Demers & Weatherhead, 2002)+
<i>Crotalus atrox</i>	Snake	1.40	(Landreth, 1973)
<i>Crotalus scutulatus</i>	Snake	1.40	(Landreth, 1973)
<i>Junco hyemalis oregonus</i>	Bird	~5	(Liebgold et al., 2013) +
<i>Masticophis flagellum</i>	Snake	0.65	(Johnson et al., 2007)
<i>Peromyscus leucopus</i>	Mouse	0.03	(Krohne et al., 1984)
<i>Pituophis catenifer</i>	Snake	0.73	(Bishop et al., 2016)
<i>Plantago ovata</i>	Grass	600	(Iwanycki Ahlstrand et al., 2019)
<i>Quercus ellipsoidalis</i>	Tree	1.57	(Axer et al., 2021)
<i>Quercus rubra</i>	Tree	1.57	(Axer et al., 2021)
<i>Rhinocelivus lecontei</i>	Snake	~1–10	(Blouin-Demers & Weatherhead, 2002)+

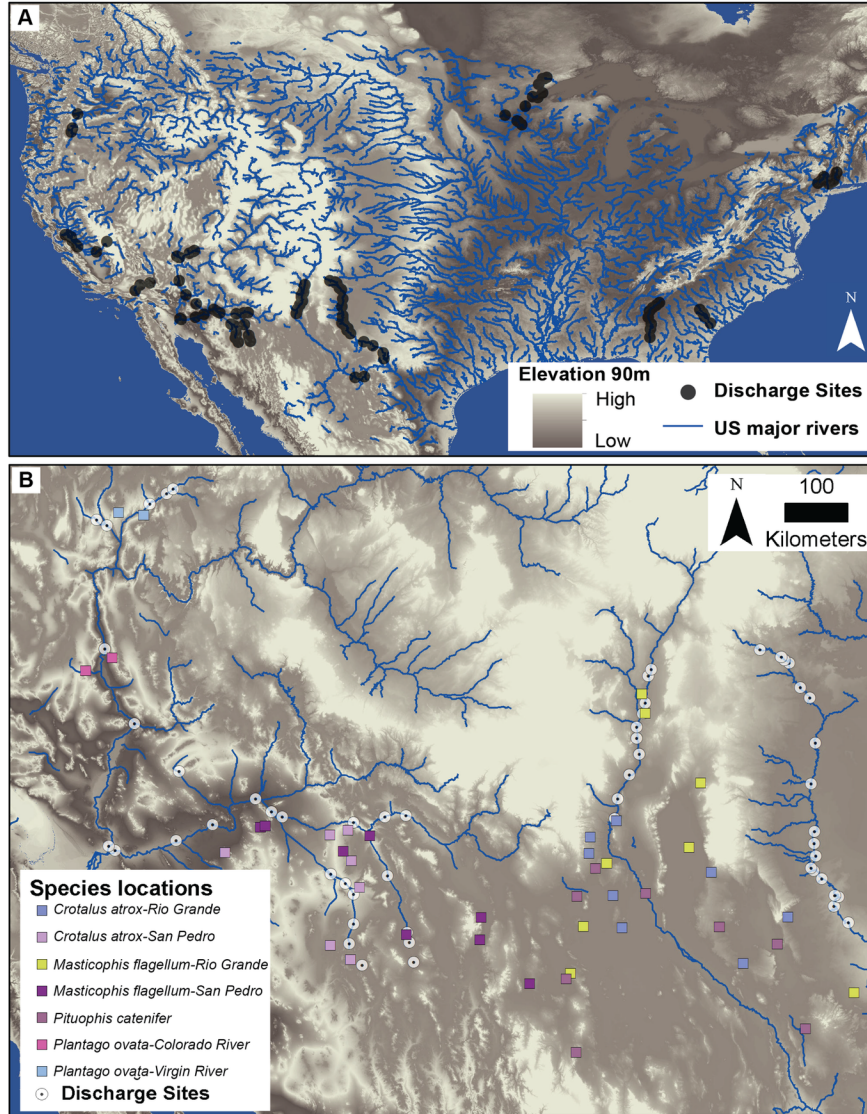
**Table 5. River data** for each river and river segment used in this study. River segments are sections of a river paired to a specific genomic dataset; some genomic datasets spanned different portions of the same river, these are indicated by a suffix after the hyphen. Both measures of seasonality (ratio and range over mean, ROM) are reported, along with standard errors (SE).

Name	Average Daily Discharge $\pm$ SE	Seasonality Ratio $\pm$ SE	Seasonality ROM
Chattahoochee	7730 $\pm$ 92	0.37 $\pm$ .01	1.52
Colorado-elegans	7040 $\pm$ 77	1.03 $\pm$ 0.03	0.90
Colorado-ovata	11900 $\pm$ 79	1.49 $\pm$ 0.01	0.86
Croton	201 $\pm$ 5	0.47 $\pm$ 0.03	1.93
Deschetes	3270 $\pm$ 35	0.81 $\pm$ 0.02	0.52
Gila	88 $\pm$ 3	1.01 $\pm$ 0.06	2.29
Housatonic	1610 $\pm$ 29	0.64 $\pm$ 0.03	1.74
Kawishiwi	355 $\pm$ 9	0.85 $\pm$ 0.03	3.45
Kettle	833 $\pm$ 8	0.90 $\pm$ 0.08	3.44
Knife	81 $\pm$ 5	0.70 $\pm$ 0.10	4.43
Mississippi	13300 $\pm$ 215	0.85 $\pm$ 0.03	2.27
Mojave	8 $\pm$ 2	0.04 $\pm$ 0.01	5.91
Muddy	43 $\pm$ 0	0.91 $\pm$ 0.01	0.27
Nemadji	423 $\pm$ 23	0.64 $\pm$ 0.07	3.38
Rio Grande- atrox	573 $\pm$ 8	1.02 $\pm$ 0.02	1.74
Rio Grande- catenifer, scutulatus	415 $\pm$ 11	3.98 $\pm$ 0.18	2.69
Rio Grande- elegans, flagellum	614 $\pm$ 8	0.92 $\pm$ 0.02	1.86
San Juaquin	940 $\pm$ 26	0.39 $\pm$ 0.02	1.50
San Pedro	26 $\pm$ 3	5.61 $\pm$ 0.62	6.44
Santa Cruz	30 $\pm$ 2	2.60 $\pm$ 0.32	3.25
Savannah	11800 $\pm$ 109	0.41 $\pm$ 0.01	1.45
St. Louis	1133 $\pm$ 42	0.59 $\pm$ 0.04	3.31
Pecos	69 $\pm$ 1	2.26 $\pm$ 0.05	2.55
Virgin	193 $\pm$ 5	0.29 $\pm$ 0.01	2.25
Wallkill	741 $\pm$ 17	0.38 $\pm$ 0.02	2.07

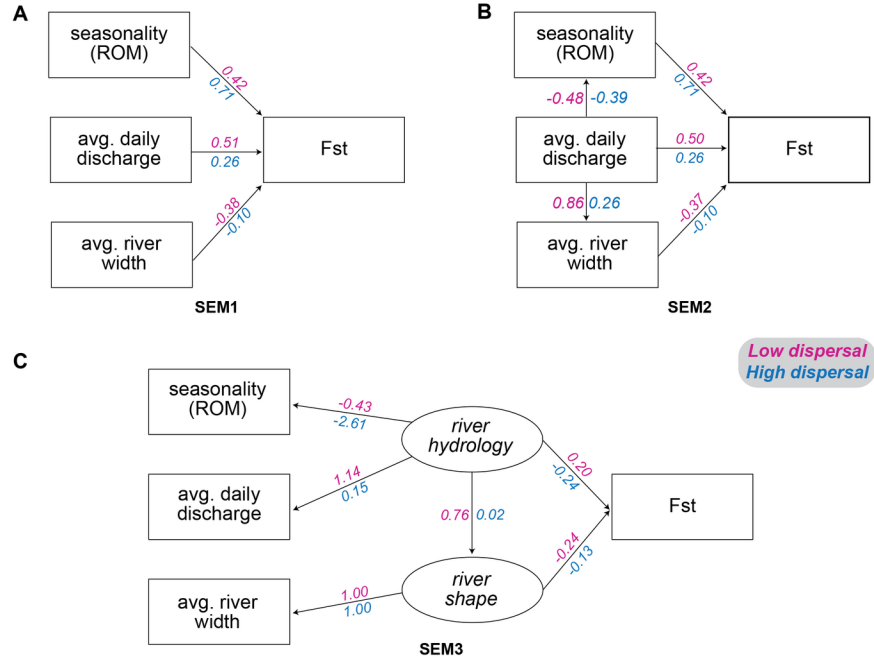
**Table 6. Summary of model results and global fit indices** for SEM models. Indices are Comparative Fit Index (CFI), Root-mean squared error of approximation (RMSEA), and Standardized root-mean Squared

Residual (SRMR) and degrees of freedom (DF) are reported 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 a good threshold for CLI (Xia & Yang, 2019). A p-value greater than 0.05 considers the model fit for the Chi-square goodness of fit test. SEM1 and SEMd1 have zero degrees of freedom (are saturated) and therefore the models of fit are artifactual. SEM2 is the best-fit model in this study. Values that meet the threshold for good model fit are bolded.

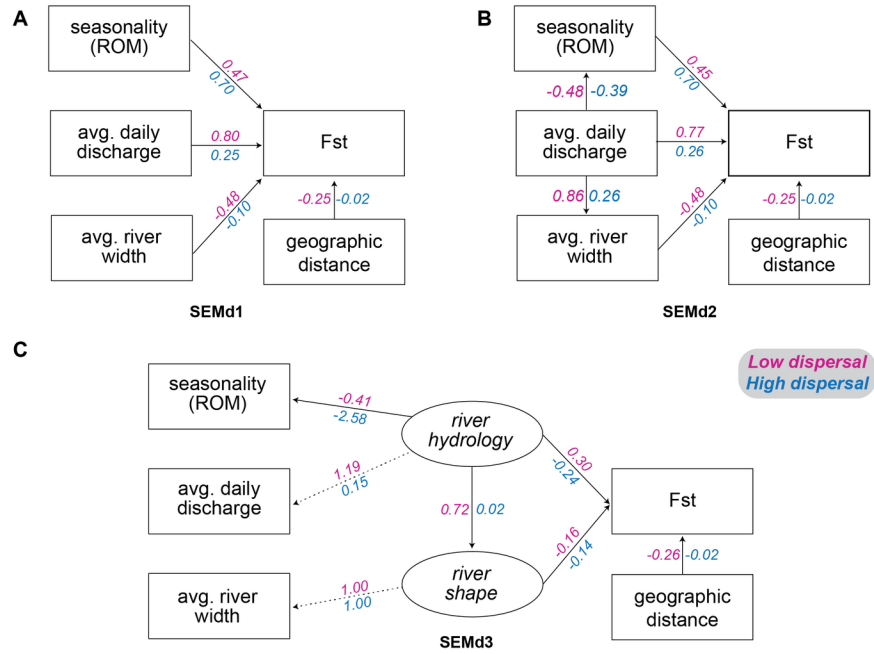
Model	DF, S / B	Dataset	Chi-Square P-Value	CFI	RMSEA	SRMR
SEM1	0 / 3	Low Dispersal	0.00	1.00	0.00	0.00
		High Dispersal	0.00	1.00	0.00	0.00
<b>SEM2*</b>	1 / 6	Low Dispersal	<b>0.59</b>	<b>1.00</b>	<b>0.00</b>	<b>0.02</b>
		High Dispersal	<b>0.86</b>	<b>1.00</b>	<b>0.00</b>	<b>0.02</b>
<b>SEM3</b>	1 / 6	Low Dispersal	<b>0.16</b>	<b>0.96</b>	0.25	0.10
		High Dispersal	<b>0.36</b>	<b>1.00</b>	<b>0.00</b>	0.09
SEMd1	0 / 4	Low Dispersal	0.00	1.00	0.00	0.00
		High Dispersal	0.00	1.00	0.00	0.00
<b>SEMd2</b>	3 / 9	Low Dispersal	<b>0.54</b>	<b>1.00</b>	<b>0.00</b>	<b>0.05</b>
		High Dispersal	<b>0.18</b>	0.67	0.23	0.16
<b>SEMd3</b>	4 / 10	Low Dispersal	0.00	0.65	0.42	0.28
		High Dispersal	<b>0.22</b>	0.64	0.19	0.18
<b>SEMd2-alt</b>	3 / 10	Low Dispersal	<b>0.04</b>	0.84	0.34	0.09
		High Dispersal	<b>0.19</b>	0.64	0.22	0.16



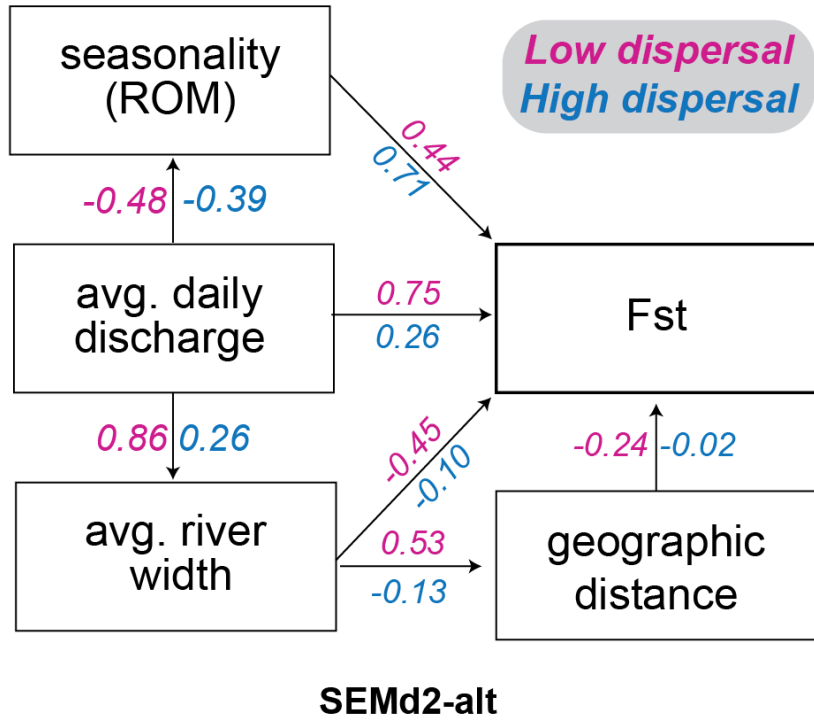
**Figure 1. Map of study area and river networks.**(A) Locations of rivers sampled in this study are shown through discharge sites. (B) An example set of rivers from the southwest with associated individuals within population pairs (squares) and discharge sites. River width was calculated at five locations near each discharge site and averaged. Not all species are plotted in this area to ease visualization.



**Figure 2. Results of the structural equation models (SEM1–3) without geographic distance.** Standardized path coefficients are shown for the low dispersal (pink) and high dispersal (blue) datasets. Circles represent latent variables, rectangles represent manifest (measured) variables.



**Figure 3. Results of the structural equation models (SEMd1–3) accounting for geographic distance within the structure.** Standardized path coefficients are shown for the low dispersal (pink) and high dispersal (blue) datasets. Circles represent latent variables, rectangles represent manifest (measured) variables.



**Figure 4. 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. Standardized path coefficients are shown for the low dispersal (pink) and high dispersal (blue) datasets.