Quantifying Diversity, Connectivity, and Environmental Resistance in Dendritic Ecological Networks

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ABSTRACT(250)

Statistical methods that quantify individual movements have limited applicability within dendritic ecological networks (DENs), to include riverscapes. We promote an approach herein by deriving an analytical framework (=DISTNET and RESISTNET) that more appropriately quantifies differentiation by meshing individual DEN segments with fitted distances and annotated environmental features. We first explored the spatial arrangement of diversity in a river network by fitting pairwise distances against graph edges. We then derived effective resistance models (e.g., as a composite of an array of possible environmental covariates) by using a genetic algorithm for heuristic model selection, invoking circuit theory (CIRCUITSCAPE) to form a graph-based complement to RESISTANCEGA (a landscape ecology package). Although numerous pairwise distance metrics can be employed, we utilized genetic distances encompassing 13,218 ddRAD loci from *N*=762 Speckled Dace (Cyprinidae: *Rhinichthys osculus*) sampled at 78 Colorado River localities (μ=9.77/site). When distances for each locus were fitted into the network, we found intraspecific divergences due to stream reaches were greater than expected when distances alone. In fitting model-averaged 'effective resistance' models to the same network, we showed the manner by which a host of anthropogenic and environmental variables contribute to patterns of connectivity underlying riverscape genetic differentiation. Our framework represents a contemporary approach to deriving metapopulation/ metacommunity structure within DENs, in that it allows: (a) The extension of projections into unsampled temporal/spatial components; (b) Comparisons to be made among species and/or drainages, both of which benefit multi-species management; and (c) the quantification of locus-specific patterns, from which adaptive hypotheses could then be derived.

"The intimate relation of hydrological conditions to fish life is proverbial; to be hopelessly or dangerously maladjusted to one's environment is to be a 'fish out of water'" (Hubbs 1941).

1. INTRODUCTION(1352)

The physical structure of watersheds not only defines the form and function of freshwater fishes (Douglas and Matthews 1992, Jackson et al. 2001), but also their intraspecific evolutionary trajectories and population dynamics (Mussmann et al. 2020b). Despite similar approaches, the resulting patterns of diversification differ markedly from those found in terrestrial landscapes and 'seascapes' (Martinez et al. 2018, Manel et al. 2020, Rabosky 2020). This is because the unique constraints found in riverine networks not only serve to define dispersal pathways but also as a template that dictates genetic relatedness within/ among aquatic biota (Meffe and Vrijenhoek 1988). Network 'connectivity' (=potential for movement) and 'complexity' (=branching rates/ nonlinear topology) thus represent constraints that control numerous population processes, such as: Genetic divergence (Hopken et al. 2013), colonization probabilities (Labonne et al. 2008, Falke et al. 2012), and ephemerality of metapopulation components (Fagan 2002, Campbell Grant 2011).

In general, complex dendritic networks may promote intraspecific genetic diversities (Thomaz et al. 2016), and divergences among distal (e.g., headwater) populations (Chiu et al. 2020a). Yet, the unidirectional flows in riverscapes differ from other such dendritic ecological networks (DENs), and thus acts to bias the patterns of dispersal found therein (i.e., up- *versus* downstream). This in turn promotes asymmetric gene flow and a source-sink metapopulation structure (Campbell Grant et al. 2007). Consequently, a prevailing trend is for a downstream increase in allelic diversity (Paz-Vinas and Blanchet 2015, Paz-Vinas et al. 2015, Blanchet et al.

2020), whereas unique genetic variation is most often retained within more peripheral headwater populations (Morrissey and De Kerckhove 2009, Chiu et al. 2020b).

Conversely, metapopulations reflect low N_e values (Lowe and Bolger 2002, Altermatt and Fronhofer 2018, Shen et al. 2018) and reduced genetic variability (Thomaz et al. 2016) within unimpeded linear systems that, in turn, impacts both resilience and stability of lineages (Mari et al. 2014, Seymour et al. 2015, Terui et al. 2019), as well as their capacity to proliferate (Harvey et al. 2019). However, numerous labile conditions can also serve to alter these expectations, such as: Life histories [e.g., directionality of dispersal bias (Chiu et al. 2020a)], nonequilibrium population processes (Pilger et al. 2017), geomorphic and climatic regimes (Douglas et al. 1999, Osborne et al. 2014), and abiotic species-level interactions (Paz-Vinas et al. 2015, Rodríguez-González et al. 2019) .

It is thus not surprising that a singular evaluation of stream distances rarely captures intraspecific genetic patterns. For example, in a comparison of 12 salmonid species, the R^2-value for isolation-by-distance (IBD) varied from 0.06–0.87 (μ=0.51; σ=0.26; Bradbury and Bentzen 2007), and from 0.0001–0.44 among three species of darter (Camak and Piller 2018). Although such deviations are partly driven by stochastic or nonequilibrium processes (Hutchison and Templeton 1999), their residuals should also reflect those spatial features that impede individual movements (Keis et al. 2013, Tang et al. 2020). Thus, divergence can alternatively be considered as a function of the movement-cost along stream segments, i.e., ‘isolation-by-resistance’ (IBR: McRae, 2006; McRae & Beier, 2007).

The IBR model applied within dendritic networks emphasizes specific attributes of individual edges or stream segments, and an array of localized factors have been identified as contributing to resistance (Hughes et al. 2009, Fullerton et al. 2010). These include: Rate of

elevational change (Lowe et al. 2006), flow regime (Rolls et al. 2016), channel width/ catchment area (Ma et al. 2020), water composition (Fourtune et al. 2016), and seasonal hydroclimatic fluctuations (Brauer et al. 2016, 2018). In addition, organisms that never (or but rarely) occupy upstream or headwater conditions can still be directly affected by those habitats (Frissell et al. 1986, Nadeau and Rains 2007, Fullerton et al. 2010).

Most linear streams are also inherently predisposed to fragmentation (Fagan 2002, Fagan et al. 2002, Brauer and Beheregaray 2020), a situation particularly exacerbated in the Anthropocene such that relatively few now remain 'free-flowing' (Grill et al. 2019). Water storage also impacts habitat suitability (Chafin et al. 2019, Fraser et al. 2019), and also shifts population trajectories (Dibble et al. 2020). Dams and diversions either extirpate populations (Brauer and Beheregaray 2020), or disrupt homing behaviors so as to promote homogenization (Baggio et al. 2018), with species of limited mobility particularly impacted (Coleman et al. 2018). Again, vulnerability and the specific nature of impacts vary by context and species (Prunier et al. 2018).

1.1 | *Applying landscape genetic methods to riverscapes*

The mechanics of landscape genetics/genomics often translate poorly to dendritic networks, and this in turn creates a disparity with regard to the number of 'riverscapes' analyzed (Davis et al. 2018, Grummer et al. 2019). At issue is the explicit consideration of riverscape structure upon which genetic relationships can be mapped. Frequent *ad hoc* approaches often employ (a) methods that lack a spatial framework (such as STRUCTURE; Pritchard et al. 2000), or (b) procedures commonly applied in landscape genetics, such as matrix dissimilarities (e.g., Kanno, Vokoun, & Letcher, 2011; Cooke, Landguth, & Beheregaray, 2014; Galbraith, Zanatta,

& Wilson, 2015). The latter, while capable of testing spatio-genetic associations, does so by contrasting a genetic distance matrix against pairwise differences in local conditions (e.g., Mantel test and derivatives; Douglas and Endler, 1982; Smouse, Long, & Sokal, 1986). These are controversial methods, in that the space separating sampling points is generally not evaluated (Legendre and Fortin 2010; Legendre et al. 2015; Peterman and Pope 2020). For riverscapes, this equates to those attributes of stream segments through which migrants must travel (Davis et al. 2018).

On the other hand, path-based methods consider the cumulative costs of movement among points, under the premise that multi- or omni-directional movements are available (an assumption that may not apply within riverscapes). Those environmental features encountered during movements are subsequently converted into ‘resistance values’ (Zeller et al. 2012), with the resulting ‘surface’ allowing for habitat-related metrics to be derived, such as: Least-cost paths, net flux between points, and movement corridors (Shah and McRae 2008, McRae et al. 2016). Operationally, this involves summarizing spatial variables as a raster image of n x m cells (analogous to pixels), with numerical resistance values assigned so as to form an 'effective resistance network' (Davis et al., 2018). Here, riverscapes transition into a graph structure, with nodes (i.e., populations or junctions) connected by edges annotated with environmental attributes (Eros et al. 2011, Peterson et al. 2013).

Few approaches have considered resistance within a spatially explicit graph-theoretic framework, and even fewer have done so by involving multiple covariates in an hypothesis-testing framework [although see White, Hanks, & Wagner (2020)]. Here, it becomes necessary to employ an heuristic approach that explores complex parameter space, particularly given the sensitivity of resistance models to parameterization and the difficulties with the *a priori*

weighting of specific variables within a larger pool of candidates (Spear et al. 2010). Such approaches have been developed for landscapes, using model exploration and selection algorithms (RESISTANCEGA: Peterman, 2018) successful in both empirical and *in silico* scenarios (Peterman et al. 2019, Kimmig et al. 2020, Winiarski et al. 2020).

Here, we employ resistance model perspectives as a mechanism to quantify riverscape genetics/genomics, and do so by exploiting recently developed, large-scale efforts that quantify/classify stream variables (McManamay et al. 2018, Grill et al. 2019, Linke et al. 2019, Troia and McManamay 2020). Our approach modifies those methods that fit distances to network edges with others that subsequently optimize graph-based resistance models (Kalinowski et al. 2008).

We utilized our package to evaluate a case study involving Speckled Dace (SPD; Cyprinidae, *Rhinichthys osculus*), the most widely-distributed species within the most endangered and regulated river system of North America, the Colorado River (Minckley and Deacon 1968, Nilsson et al. 2005, Grill et al. 2019). To accomplish this, we analyzed a large ddRAD dataset as a means to test the following hypotheses: 1) SPD populations are structured according to contemporary riverine connectivity; 2) Their genetic diversity is driven by the topology of the dendritic network; 3) Genetic differentiation is modulated by environmental attributes, represented as edges within the river network; and 4) Spatio-genetic patterns are not explained by distance alone but rather by an isolation-by-resistance model.

2. METHODS (3098)

2.1 | *The automation of population genetic analyses in dendritic networks*

Two complementary software tools, DISTNET and RESISTNET, facilitate high-throughput analyses by employing two complementary software tools that automate the exploration of

environmental correlates as well as the analyses of genetic differentiation in dendritic networks (i.e., DISTNET, RESISTNET; Fig. 1). Both are available as open-source which also includes instructions for virtual deployment as well as installation from scratch (github.com/tkchafin/DENdriscape).

2.1.1 | *The fitting of edge-wise genetic distances*

The DISTNET workflow (Fig. 1) parses geospatial input files (as *.shp*) representing large stream networks in which streams=edges, and endpoints or junctions=nodes. It does so by: 1) 'snapping' sample points to nodes in the river network; 2) Identifying shortest paths between each set of points using Dijkstra's algorithm (Dijkstra 1959), (as implemented in NETWORKX; Hagberg et al. 2008); and 3) Constructing a minimized network representation, preserving original geometries, forming contiguous edges by joining individual stream reaches/ segments, and retaining only those junctions necessary to fulfil shortest Dijkstra paths between points. DISTNET will annotate final results as part of the original geospatial dataset. Given this, we recommend employing the RIVERATLAS dataset (hydrosheds.org/page/hydroatlas) that contains pre-calculated variables representing various aspects of local-scale hydrology (such as physiography, climate, land-cover, geology, and anthropogenic effects) (Linke et al. 2019). This not only simplifies downstream analysis (as herein) but also facilitates visualization of results using graphical geospatial software such as ARCGIS (Esri, Inc.) or QGIS (www.qgis.org).

DISTNET will then calculate pairwise measures of genetic differentiation and stream distances among individuals, sites, or populations. For individual-based metrics, we implemented both uncorrected *p*-distances (i.e., proportion of nucleotide differences over the alignment length), as well as corrected measures [e.g., TN93 (Tamura and Nei 1993)]. Several frequency-

based metrics can also be implemented for population- or site-wise calculations, as well as whole-locus single alignments, multi-locus alignments, or SNP datasets. These are: θ_{ST} (Weir and Cockerham 1984); G'_{ST} (Hedrick 2005); G''_{ST} (Meirmans and Hedrick 2011); Linearized F_{ST} (Rousset 1997); Jost's D (Jost 2008); Chord distance (Cavalli-Sforza and Edwards 1967), as well as others (github.com/tkchafin/DENdriscape). For more efficient computation when very large numbers of SNPs are employed (as herein), we also recommend that locus-wise distances be pre-calculating using ADEGENET (Jombart 2008) Scripts again available at: github.com/tkchafin/DENdriscape/scripts.

Although isolation-by-stream-distance methods are available in DISTNET, the primary approach is the Kalinowski et al. (2008) model in which a least-squares optimization procedure (Felsenstein 2004) is utilized to fit branch lengths. Here, the procedure begins with a matrix of pairwise genetic distances, then seeks to map those stream distances as explained by k segments in a network, such that the fitted distance values (r) for each segment separating two populations will sum to the observed value from the pairwise matrix. The result is a distance (r_k) for each of k segments that represents the genetic distance 'explained' by that segment (Fig. 1; see also Kalinowski et al. 2008).

The underlying mathematical implementations include an iterative, constrained optimization approach that corrects for negative-fitted distances (per Kalinowski et al., 2008). However, we provide an additional opportunity for alternative weighting within the least-squares optimization (e.g., Fitch & Margoliash, 1967), that will alter the weight of distances based upon their similarity, which may in turn be beneficial when rates of change vary among branches (or in this case, stream edges; Felsenstein 2004). In addition, the original method (Cavalli-Sforza and Piazza 1975) also remains available for subsequent implementation.

Fitted distances can either be computed either for a single locus, as an aggregate (e.g., arithmetic mean) across loci. The result is a table of fitted distances per stream segment (and per-locus, if so requested), as well as an annotated version of the original geospatial data. Each can be employed for downstream analysis, as demonstrated in our case study. Interestingly, DISTNET can be re-calculated using graphs previously constructed as input (i.e., as a programmatic option).

2.1.2 | *Composite resistance models optimized within networks*

RESISTNET combines a genetic algorithm with multiple environmental variables so as to explore the parameterization of resistance models (Fig. 1), similar to a process found in RESISTANCEGA (Peterman 2018), but with several important differences. For example, genetic algorithms are useful in modelling optimization and selection of features in that they are less impacted by local optima when compared to other 'hill climbing' techniques (Prügel-Bennett 2004). They are employed to simultaneously evaluate a 'population' of models by using log-likelihood or AIC to gauge 'fitness.' Here, 'individuals' (as randomly sampled model configurations) consist of 'chromosomes,' with 'genes' (as parameter choices) being represented by: 1) Variable inclusion/exclusion; 2) Variable transformations (using transformation functions available in RESISTANCEGA; Fig. S1); 3) Shape parameters for transformation; or 4) Variable weights (with user-defined ranges).

For an optimization problem involving *n*-environmental parameters, an individual chromosome is represented as length=4*n*. Fitness values (e.g., AIC) then determine the survival of individual models and their contribution to successive generations, with stochasticity represented by mutation (random changes to parameter values) and recombination (parameter

choices from multiple individuals combined as offspring). Optimization continues until a threshold number of generations have passed without the fitness of the population increasing (Scrucca, 2013).

The optimization process is summarized as follows (with the DEAP evolutionary algorithm package enabling implementation; Fortin et al. 2012):

1. Network structure and environmental data are imported, using the output formats of DISTNET. Environmental data for contiguous stream reaches (=edges) are summarized and rescaled from 0–10, following Peterman (2018).
2. The initial population is randomly seeded, with a user-defined population size. Individuals are composed chromosomes of length *n**4, where *n*=the number of environmental variables (as above). Transformation, shape, and weight parameters ensure that an optimal scale can be 'found' with respect to a potential non-linear relationship between gene flow and each variable, while also allowing the latter to vary in a composite resistance modelwith regards to relative influence and direction (e.g., positive or negative).
3. Individual fitness is calculated using a custom function, wherein all selected variables are transformed according to the chosen parameters, then re-scaled and combined using a weighted additive function to form a single composite set of resistance values that annotate the original network. Cumulative effective resistance between nodes is then computed using CIRCUITSCAPE. Final calculations employ a maximum likelihood population effects (MLPE) linear mixed effects model to compare pairwise genetic distances with scaled and centered resistance distances, fitted using the MLPE function from RESISTANCEGA (via the RPY2 interface; Gautier 2008). Fitness is then returned via

options available in RESISTANCEGA as either log-likelihood, AIC, or marginal R^2. Fitness can also be computed using ΔAIC_{null}, or the change in AIC values via the MLPE-fitted model, as compared to a 'null' model composed of only random effects.

4. Individuals are 'selected' for successive generations using a tournament selection scheme, a deterministic method whereby random sets of individuals of size *n* specified by the user are compared (=tournaments), with 'winners' being retained.
5. Mutation (=changing parameter values) and recombination (=exchanging parameter combinations among models) occur at generational intervals, with the respective probabilities of both processes as user-specified.
6. Steps 2-5 are repeated for a user-specified number of generations, or until a number of user-specified generations fail to increase the maximum fitness beyond a given threshold.
7. After stopping criteria are met, Akaike weights are computed for each of the 'top' models observed in the population, with models retained so as to meet a cumulative user-specified Akaike weight threshold (default value=0.95). Model-averaged resistance and multi-model, relative variable importance values are subsequently computed using the chosen model set (below).

Multi-model inference has several benefits: It avoids the inherent subjectivity of model selection, in that the estimates from every candidate model are integrated proportional to their approximation of the observed genetic distances (using the Akaike weight; w_i for *i* models), while also more concisely incorporating both uncertainty and information from closely-ranked models. Additionally, it also allows relative variable importance (RVI) to be quantified across models composed of different numbers of explanatory variables, and does so by deriving the sum

of Akaike weights that correlate well with *in silico* evaluations of r^2 variable rankings (Giam and Olden 2016).

2.2 | Rhinichthys osculus *as an empirical case study*

As a study species, we focus on the small-bodied SPD, broadly endemic to western North America and represented within all major drainage basins west of the Rocky Mountains. Its distribution serves as a biodiversity bookmark for hydrography and geomorphology of western North America (Hubbs and Miller 1948, Minckley et al. 1986, Oakey et al. 2004, Smith and Dowling 2008). As such, it is remarkable adaptive, extending from high-altitude/ cold-water streams of the Pacific Northwest (Pfrender et al. 2004, Hoekzema and Sidlauskas 2014), to sub-sea level endorheic basins in the Mojave/ Sonoran deserts (Mussmann et al. 2020b). Extreme heat, aridity, and salinity have not only extended its physiological limits but also diversified its phenotypic evolution (Kaya et al. 1992, Lema 2014, Lema et al. 2019).

This extraordinary phylogenetic diversity also encompasses highly restricted habitats that are vulnerable to stochastic and anthropogenic influences (Mussmann et al. 2020b). Consequently, and as validated by history, it remains vulnerable to extinction, with two lineages being contemporaneously eliminated in the 20th century, and four subspecies listed under the United States Endangered Species Act of 1973, as amended (16 U.S.C. 1531-1544, 87 Stat. 884). This contemporary decline and extirpation underscores a vulnerability to ongoing climate change (Minckley and Deacon 1968), an hypothesis we test herein by examining several summary indices of anthropogenic modification, in addition to an array of environmental and hydrological predictors (Table 1, and descriptions below).

2.2.1 | *Sampling and data generation*

Fin tissues were collected for *N*=762 individuals distributed throughout 78 Colorado River Basin localities (μ=9.8/site) (Fig. 2; Table S1; Mussmann, 2018). Whole genomic DNA extractions involved several methods (i.e., Gentra Puregene DNA Purification Tissue kit, QIAGEN DNeasy Blood and Tissue kit, QIAGEN QIAamp Fast DNA Tissue Kit, or CsCl-gradient extraction). These were subsequently quantified via Qubit fluorometer (Thermo Fisher Scientific, Inc.) and assessed via gel electrophoresis (2.0% agarose). Sequencing followed the ddRAD protocol (Peterson et al. 2012), with restriction digest of 1µg DNA per sample using *Pst*I and *Msp*I enzymes (New England Biolabs) at 37ºC for 24 hours, followed by bead-purification (Agencourt AMPure XP; Beckman Coulter, Inc.). Samples were standardized (100µg each), ligated with uniquely-barcoded adapters, then pooled into sets of *N*=48/ library. These were size selected (375-425bp; Sage Science Pippin Prep), then extendedn by PCR-based adapter (x12 cycles) using Phusion high-fidelity DNA polymerase (New England Biolabs). Three uniquely-indexed libraries (=144 samples total) were pooled per 1x100 base pair (bp) lane for sequencing on the Illumina HiSeq 2000 (University of Wisconsin Biotechnology Center) or HiSeq 4000 (University of Oregon Genomics & Cell Characterization Core Facility).

2.2.2 | *Data assembly and filtering*

Reads were demultiplexed and assembled using the *process_radtags* module (STACKS V2.41; Rochette et al. 2019), followed by elimination of low-quality reads (Phred quality score <20), uncalled bases, and missing restriction cut sites, while allowing one barcode mismatch. Assembly was performed using *de novo* clustering in STACKS, with samples hierarchically grouped into *N*=5 subregions: Upper Colorado River Basin (*N*=221), Virgin River (*N*=223),

Grand Canyon and tributaries (*N*=149), Gila River Basin (*N*=105), and Little Colorado River (*N*=64). This ensured recovery of informative loci within each region. Trial runs using STACKS yielded optimal assembly parameters (Paris et al. 2017) for the minimum number of raw reads necessary to form a stack (m=4), as well as the number of mismatches between stacks for identification of putative loci (M=2), and catalog formation (n=2). The *populations* module was utilized to identify SNPs shared by individuals located within a subregion, as outlined above. Loci appearing in <50% of individuals from each subregion were removed, as were loci with an observed heterozygosity >0.6, and a minor allele frequency (MAF) <0.01. These measures removed putative paralogs and minimally informative loci (Eaton 2014; McKinney et al. 2017).

2.2.3 | *SPD population structure*

To provide context for our analyses of riverscape resistance and genetic differentiation, we first gauged population structure by employing an aspatial clustering method (ADMIXTURE; Alexander et al. 2009). Data were filtered to one SNP/ locus, with samples partitioned by region. To do so, we evaluated models containing *K*=1-20 possible sub-populations across 20 replicates, executed in parallel (ADMIXPIPE pipeline; Mussmann et al. 2020a). Cross-validation (CV) values were calculated in ADMIXTURE and summarized across replicates.

Replicates were clustered and evaluated in CLUMPAK (Kopelman et al. 2015) so as to identify potentially discordant modes within a single *K* value. Major clusters were identified using a threshold of 90%. Optimal *K* represented the lowest CV value.

2.2.4 | *Environmental and anthropogenic variables*

To test environmental correlates of genetic differentiation, we examined 26 continuous environmental and anthropogenic variables (see Table 1) per stream reach, as parsed from global databases (Grill et al. 2019, Linke et al. 2019). Additional variables were removed from the referenced databases if they exhibited a significant pairwise correlation coefficient (Pearson's *r*) >0.7 (per Holm's multiple test correction; Holm 1979).

2.2.5 | *Environmental associations and riverscape resistance*

We first calculated pairwise linearized F_{ST} (=F_{ST}/1- F_{ST}) for each sampled SNP locus (ADEGENET; Jombart 2008). These were input into DISTNET using the *--locmatdir* and *-r RUNLOCI* options. Samples were grouped by locality and, to maximize comparability with STREAMTREE estimates (Kalinowski et al. 2008), the unweighted least-squares approach (Cavalli-Sforza and Edwards 1967, Kalinowski et al. 2008) was employed to fit our stream trees. We used the *--iterative* argument to correct for negative distances. For the input stream matrix, we used a stream layer representing all of North America, as clipped from the RIVERATLAS dataset (Linke et al. 2019). This was subsequently reduced to a minimal contiguous sub-network by an automated procedure performed within DISTNET.

Output included a geodatabase and shapefile that mapped contiguous-edge IDs with the original segment identifiers from RIVERATLAS (=HYRIV_ID), as well as fitted F_{ST} values for each locus assigned to each edge, and a mean/ standard deviation across all loci. We performed an automated generalized linear mixed model selection procedure (GLMULTI; Calcagno and de Mazancourt 2010) so as to first examine the relationship between the mean fitted distances and the underlying environmental RIVERATLAS features (Table 1). A genetic algorithm was used to explore all possible sets of explanatory variables, including pairwise interactions among

variables. RVI values were computed using the sum of Akaike weights. Prior to analysis, all variables were scaled, centered, and transformed to approximate normality (BESTNORMALIZE; Peterson and Cavanaugh 2019). To consider the effect of variable dispersion, a second GLMULTI run examined the coefficients of variation (c_v) for raw values, calculated per network edge as the ratio of the standard deviation among all contiguous stream reaches composing an edge by the absolute value of the mean.

We then performed redundancy analysis (RDA), with the number of variables reduced using a forward selection procedure with 10,000 permutations [ADESPATIAL; (Dray et al. 2018)]. The iterative selection procedure was halted using an adjusted R^2 threshold computed from a global RDA using all variables (VEGAN; Oksanen et al 2007). A partial RDA (pRDA) conditioned on edge lengths, was employed to 'partial-out' the IBD effects on F_{STs} fitted edge-wise. Significance was evaluated using the *anova.cca()* function in VEGAN, with outliers identified as stream edges or SNPs having loadings +/- 3 standard deviations from the mean, and labelled according to the predictor variable having the greatest correlation.

The above methods examined the correlation of fitted distances *versus* edgewise environmental variables in the stream network. As contrast, we also applied the RESISTNET approach using raw values to compensate for the internal optimization, transformation and scaling of variables. Four replicates were employed, with population sizes equating to either 288 or 512. Exploratory analyses were used to tune mutation (*mutpb* and *indpb*) and crossover (*cxpb*) parameters, and to benchmark runtime. The maximum number of generations for full runs was set at 500, with a stop-criterion (=50 generations) engaged if indeed the best model (*nfail*) failed to improve. All transformation types were allowed, and variables weights ranged from 0-1. Input represented the optimized graph and outputs from DISTNET.

3. RESULTS (1414)

3.1 | *Data assembly and population structure*

The global assembly represented 13,218 loci at 25.94x mean depth of coverage, with 27.6% missing data after those loci present in <50% of individuals were removed. ADMIXTURE identified *K*=30 populations summed across the five *a priori* defined sub-basins (Fig. 3). The Upper Colorado River Basin was divided into *K*=6 groups composed of major tributaries: Chinle Wash, Green River, Paria River, San Juan River, Smiths Fork River, and Vermillion Creek, with the San Juan River encompassing the Fremont and San Rafael rivers. The Virgin River Basin was partitioned into *K*=7 sub-populations: Pluvial White River, Pahranagat River, Moapa River, Meadow Valley Wash, Beaver Dam Wash headwaters, and Virgin River headwater/ mainstem (Fig. 3). Of note, two localities contain unique SPD subspecies: Pahranagat (*R. o. velifer*), and Moapa (*R. o. moapae*).

The remaining *K*=17 sub-populations were distributed throughout the Lower Colorado River Basin, with *K*=5 being distributed linearly through the Grand Canyon (Fig. 3). The remainder were divided among the Bill Williams River (*K*=1), Little Colorado River (*K*=4), and Gila River (*K*=7). The latter was also represented by major tributaries and stream hierarchy: Agua Fria, Eagle Creek, San Francisco River, San Pedro River, San Simon River, Verde River, and Upper Gila tributaries (Fig. 3).

3.2 | *Stream hierarchy via DISTNET*

The minimal graph from DISTNET contained 114 edges representing contiguous combinations of stream reaches, with nodes either as sampling localities or the minimum number of necessary junctions required for shortest Dijkstra paths (Fig. S2). Fitted, linearized F_{ST} for many mainstem

reaches of the Colorado and Green rivers (globally over all loci; Fig. 4A) trended very low (F_{ST}=0.0-0.1). Elevated values (F_{ST}>0.2) were more common in the Lower Colorado River Basin. For example, the Agua Fria and Bill Williams rivers both had values >0.35, with higher values also found in multiple smaller tributaries (e.g., Salt River >0.25; Fig. 4A). Several inter-drainage connections also displayed elevated values: Grand Canyon–Virgin River (>0.15); Grand Canyon–Little Colorado (>0.15); Lower Colorado–Gila River (~=0.10). Few in the upper Colorado River Basin tributaries had F_{ST}>0.1, save for the North Fork of Vermillion Creek in Wyoming (>0.25).

Linearized F_{ST} for all SNP loci averaged across DISTNET (via *-r RUNLOCI*) generally mirrored the global estimates (Fig. 4B). The connections between Little Colorado and Gila River basins were exceptions, with the Colorado River mainstem being slightly higher relative to the median. The same was observed when examining standard deviation of per-locus fitted-F_{ST} (Fig. 4C), with slightly higher relative values in the segment connecting the Virgin-Muddy Basin with the Colorado River in the Grand Canyon.

The majority of sampling localities were within 0-2km of nodes in the input stream network, suggesting scant effect due to 'snapping' error or missing stream segments (Fig. 4D). The fit between predicted distances *versus* input F_{ST} was similar to that calculated in the original STREAMTREE program (adjusted R^2=0.77 versus 0.787), again suggesting that slight differences in implementation produced minimal error (Fig. 4E). Stream distances alone were also a relatively poor predictor of F_{ST}, particularly when compared with those predicted using the STREAMTREE model (Mantel r=0.47, p<0.001; Linear model R^2=0.22, p<0.01; Fig. 4F).

3.3 | *Environmental covariates and riverscape resistance*

3.3.1 | *Testing correlates of fitted distances*

Generalized linear model (GLM) selection evaluated edgewise variable means (μ) and six variables [via variation coefficients (c_v) via GLMULTI (Fig. 5B, Table 2)] so as to identify eight variables at a model-averaged RVI threshold of 0.8 (Fig. 5A, Table 2). Two variables (i.e., USE and TMP) exceeded >0.8 when both μ and c_v were considered. Analysis of variable μ_s also implicated three anthropogenic (POP, HDI, CSI), two hydrologic (RES, DOF), and one land-cover variable (PAC). Additionally, model-averaged RVI for c_v implicated ARI, SOC, and RIV as well (Fig. 5A, Table 2).

Global RDA of stream-wise means were statistically significant (p<0.001, adjusted R^2=0.127). Forward selection evaluated a minimum number of maximally discriminatory variables and chose 10 that met the adjusted R-threshold. Implementing a correction for false-discovery rate (FDR) via the Benjamini-Hochberg procedure resulted in four variables being selected: AET, PET, USE, and DOF (Fig. 5B-E, Table 2). VIFs ranged from 1.19-1.88, and both RDA and partial RDA were significant for the selected variables (p<0.001) (with pRDA conditioned on LENGTH). The pRDA designated 221 SNPs (Figs. B-C) and 9 edges (Figs. D-E) as outliers, with the largest proportion having the strongest direct correlations with evapo-transpiration (AET/ PET; Table S2).

Stream-wise variation coefficients from the global RDA were also significant (p=0.007, R^2=0.12), with four variables chosen via forward selection: PET, SOC, SLP, and CSI (Figs. 5F-I, Table 2). Both RDA and pRDA for the selected variables remained significant (p<0.001), with VIFs ranging between 1.04-1.21. Based on pRDA loadings, 349 SNPs (Figs. 5F-G) and eight edges were identified as outliers (Figs. 5H-I), with outlier associations most prevalent for SOC and PET (Table S2).

3.3.2 | *The optimization of resistance models*

We ran four replicate RESISTNET analyses within two different computing environments: Replicates 1 and 2 done virtually on the JETSTREAM cloud (Hancock et al. 2018), while 3 and 4 were on Linux-based high-performance compute (HPC) nodes in the TRESTLES cluster at University of Arkansas. We also varied the population size from N=288 (for 1 and 3) to N=512 (2 and 4).

All replicates running the complete SPD dataset terminated in <24 hours, with an increase in *mutpb* and *indpb* parameters that controlled the probability of individual models and parameters being selected for random mutation. This is necessary step in that rates of mutation (*mutpb* and *indpb*) and crossover (*cxpb*) will vary as a function of parameter space and population size. Here, we recommend these parameters be tuned a priori so as to promote mixing and prevent stationarity.

Parallelization was examined during exploratory runs and yielded non-linear improvement with increasing numbers of dedicated processor cores. We saw ~3.5X speed-up going from 1 to 4 processors, whereas increasing from 1 to 16 processors yielded ~10X improvement (Fig. S3). Even so, we found this to provide a substantial performance benefit over dedicating additional threads to parallel processing by CIRCUITSCAPE (Fig. S4), thus we advise complete CPU utilization should be dedicated to distributing genetic algorithm evaluations by RESISTNET (supplied using the *–procs* command-line argument).

Full analyses at N=288 or 512 met stopping criteria in ~60-70 generations and demonstrated a fairly rapid plateau in fitness (Fig. S5). The ‘best’ models in each replicate run varied in fitness, with a moderate predictive capacity as indicated in the MLPE mixed effects

models (marginal R^2=0.296-0.343). Model-averaged effective resistance networks were similar across all replicates, with streams in the Virgin, Gila, and Little Colorado River sub-basins generally having greatest resistance, with the lowest being found in upper Green River tributaries and the mainstem Colorado River above the Grand Canyon (Fig. 6A). Some smaller tributaries in the Upper Colorado River Basin also had high relative resistance values, such as tributaries of the San Juan (i.e., mainstem of Chinle Wash and the Mancos River), and tributaries of the Green River (i.e., Vermillion Creek, and the White River).

Individual replicates were also largely consistent with regard to environmental covariates that defined the composite resistance models, as selected for averaging: ARI, CRP, DOR, PAC, PET, SED, and USE in all four replicates (=100%), whereas SOC and URB were in 75% (Table 2). The remainder (i.e., AET, POP, SGR, SLP) were present in 50% of replicates (Table 2). However, those streams that failed to select catchment slope (SLP) as a covariate, did so instead with stream gradient (SGR), which we interpret as a functional equivalent. Three additional covariates (i.e., DOF, PST, RDD) were selected but once, with generally low model-averaged weights (0.08-0.42).

Model-averaged resistance values were highly correlated among replicates (Pearson's r=0.69-0.96; Fig. 6C), with Replicate 2 having the greatest discordance. Of note, this replicate converged on the highest likelihood model (Fig. S5) and also a replicate that that optimized the larger model population (N=512), potentially highlighting the necessity of a greater population size so as to identify global fitness maxima. Pairwise effective resistances among sites for each replicate were likewise highly consistent (Mantel r=0.94-0.99), with all being highly significant (p<0.0001; Fig. 6C).

4. DISCUSSION (1669)

Spatio-genetic patterns in landscape and riverscape genetics must first be defined before hypotheses can be tested regarding habitat availability, inherent barriers, and potential corridors. Yet, most methods common to riverscape genetics, for example, fail to consider network architecture (Davis et al. 2018), or those attributes that define paths through which individuals must travel. The analytical framework we provide serves to characterize the manner by which genetic diversity and network topology juxtapose, and does so by mapping multi-locus distances onto habitat edges, with causation then explored in a heuristic but rigorous manner. In so doing, we examine an arbitrary number of covariates by employing an explicit graph structure, accomplished without designating variable weighting or *a priori* candidate models.

The utility of our approach is underscored by its application in a case study involving SPD in the Colorado River Basin. For some tributaries, highly-fitted F_{ST} (Fig. 3) and high resistance in multiple sub-basins (Fig. 6) indicate the elevated divergence of isolated lineages, even within relatively well-connected riverscapes. Interestingly, segment length was often non-explanatory with regards to basin-wide genetic diversity (e.g., Table 2), implicating a loss of connectivity as more important with regard to intraspecific divergences. This, in turn, juxtaposes well with the prevalence of unique and relictual lineages within isolated watersheds (Oakey et al. 2004, Mussmann et al. 2020b).

Connectivity was likewise implicated as an important factor for consistent recovery of covariates linked to drought tolerance and seasonal persistence of streams (e.g., AET, ARI, PET). Additionally, the dominant association of these variables with outlier loci (Table S2, Fig. 5), suggests the presence of local adaptation with regard to ephemeral flow regimes. Hydrology has similarly been implicated as a driver of morphological diversification in numerous

freshwater systems (Langerhans 2008, Meyers and Belk 2014, Bruckerhoff and Magoulick 2017), and its importance in sustaining native fish assemblages has been documented across southwestern streams (Propst and Gido 2004, Probst et al. 2008, Kominoski et al. 2018). The large weights attributed to those variables that define stream persistence clearly reflect the impacts of aridity and persistent drought in the region, both of which are omnipresent across contemporary desert riverscapes (Kingsford 2006, Ruhí et al. 2015).

Covariates relating to contemporary (e.g., anthropogenic) water manipulation and stream fragmentation were also repeatedly chosen across analyses. Here, factors such as the prevalence of anthropogenic land-use categories [e.g., cropland (CRP); urban extent (UBR)], as well as multiple indices of hydrologic manipulation [e.g., water usage (USE); degree of regulation (DOR); sedimentation rate (SED)], consistently contributed to composite resistance models (Fig. 6; Table 2). Several of these are functionally-related, such as: Agricultural/ urban areas as major drivers of water usage (USE); flow regulation/ flood dampening (DOR) as controlling sediment supply (SED) as well as channel width and morphology (Grams et al. 2020, Walker et al. 2020).

Anthropogenic factors that promote habitat instability in the Colorado River also contribute significantly to the permeability of species boundaries in endemic cyprinids, with population demographic and genetic trends being impacted (Chafin et al. 2019). These altered conditions may also change the distribution and suitability of low-flow refugia, serving in turn to extirpate peripheral populations (Schlosser 1995, Bezzerides and Bestgen 2002, Magoulick and Kobza 2003). Altered conditions also promote the establishment of non-native species (Gido and Propst 1999, Whitney et al. 2017, Dibble et al. 2020), which may in turn elevate predation rates and promote competition (Marsh and Douglas 1997, Seegert et al. 2014). Predation pressure by

resident fishes also constrains the invasion success of introduced SPD (Harvey et al. 2004), thus suggesting a potential role for non-natives in promoting 'biotic' resistance.

4.1 | *Stream hierarchy and riverscape resistance models*

Our case study demonstrates both the utility and limitations of the DISTNET and RESISTNET approaches. Before each can be applied, a necessary understanding is required of how historical factors such as shifting distributions may also impact results. For example, the optimization of a resistance model will be poor when 'true' correlation among genetic and resistance distances is low (Winiarski et al. 2020). Another implicit assumption is that genetic divergences are attributable to the edges within the network (Kalinowski et al. 2008). A potential example of where this might fail could be admixture involving unsampled lineages. For example, Minckley (1973) suggested that intergradation between morphological forms of SPD in the Little Colorado and Gila river drainages was driven by historic stream capture along the Mogollon Rim, with historically-transient connections possible as well between some Upper Colorado River Basin tributaries and those of the Bear or Snake rivers (Smith and Dowling 2008, Loxterman and Keeley 2012). Historic reticulation violates an assumption of our resistance model, in that connectivity does not involve edges external to the network.

As with landscape genetic studies, individual movements via extremely rare natural connection or human-mediated translocations will also bias result by altering spatio-genetic associations (Chafin et al., 2021). However, these occurrences could potentially be adjusted for by adding additional edges as historic connections, under the assumption that *a priori* hypotheses can be formulated. Inflated genetic divergences may also emerge as vestiges of historic barriers, such as vicariant Pleistocene lava flows that separate the upstream Little Colorado River from

the Grand Canyon (Duffield et al. 2006). If indeed unrecognized, then F_{ST}-values fitted along that stretch would be incorrectly inflated. Historic population dynamics could also lead to a situation where genetic divergences fail to juxtapose with extant conditions, which may explain the high divergence displayed by Vermillion Creek, which may have inadvertently escaped large-scale, indiscriminate rotenone poisoning in the 1950s-60s as a mechanism to exterminate non-native species in the Green River (Holden 1991). Finally, it is also possible that environmental and/ or anthropogenic components of riverscape resistance may vary at smaller spatial scales, especially in those species widely-distributed. Here, a mechanism of test is provided in RESISTNET as an analysis partitioned by sub-basin. The relationship between particular covariates and resistance may often be non-linear, or instead drive resistance as a function of variance (e.g., depth or flow within a reach), rather than by per-edge means (as were examined in resistance models herein). A contrast between variation coefficients and the GLM results of edge means could lends weight to the latter. Non-linear interactions are evaluated in RESISTNET via the simultaneous optimization of variable transformation and shape parameters, yet this remains a limitation in the *post hoc* analysis of DISTNET results (as herein) and may explain why numerous covariates were implicated in the resistance models. Alternate aggregation methods (e.g., variation coefficients) could also be employed with RESISTNET, although they are not currently integrated at this point.

These limitations serve as a necessary context from which to develop additional applications, or as avenues for future research, as do emerging developments in riverscape genetics (i.e., this study and White et al., 2020). One extension for locus-wise analysis (as demonstrated herein with RDA; Fig. 5), is the identification of functional genomic associations that can promote isolation-by-adaptation rather than environmental resistance or limitation via

dispersal (Orsini et al. 2013). Adaptive genetic variation is a relatively underexplored theme in riverscape genetics (Davis et al. 2018), due largely to a dearth of methods that detect 'outlier' loci within the autocorrelative structure of dendritic networks. Thus, to this end, we hope that a broader exploration of adaptive patterns in riverscapes can be facilitated multi-locus fitting in DISTNET.

Another advantage of the RESISTNET is the potential to extrapolate and predict genetic responses in unsampled space. Here, optimized resistance models may be applied to any dendritic ecological network (to include riverscapes, as herein), so as to predict effective resistance in regions within which genetic data has yet to be obtained. This could promote *a priori* hypotheses of population structure and phylogeography that serve to predict transecting regions with elevated divergences, or inform analyses requiring assignment priors (Hubisz et al. 2009). The resulting contrasts between hypothesized and observed divergences may also target sub-drainages within which discordance has been driven by historical processes.

Optimized resistance models might also be used to predict temporal change, with models forged by contemporary data then used to extrapolate metapopulation dynamics under conditions predicted due to climate change or other disturbance (Comte and Grenouillet 2013, Comte and Olden 2017). Importantly, the approach could also be applied to any type of pairwise distance matrix. For example, DISTNET could employ community data to examine edge-wise distances or topological importance in generating differences (Eros et al. 2011), whereas RESISTNET could be used to examine how metacommunity structure is defined by environmental resistance. Again, any form of spatially structured ecological network can be generalized to accommodate either.

The potential of DISTNET and RESISTNET for increased automation also serves to encourage large-scale comparative riverscape studies involve variation among species in spatial

or temporal trends, the results of which could vary substantially as a function of trait synergism or life histories (Comte et al. 2014, McManamay and Frimpong 2015, Troia et al. 2019). While rare, such intraspecific studies have implicated ongoing evolution within dendritic networks, while testing specific ecological questions (Fourtune et al. 2016, Pilger et al. 2017, Paz-Vinas et al. 2018, Blanchet et al. 2020). For example: Why do some species exhibit greater intraspecific variation in functional/ morphological attributes than do co-occurring species? (Raffard et al. 2020). How might traits or life histories shape this contrast? How do these patterns relate to long-term macroevolutionary trends (Singhal et al. 2018, Harvey et al. 2019)? Further, a comparative framework also promotes an examination of those commonalities driving community-level structure or shared biogeographic breaks, which may in turn yield trends generalizable to conservation planning at contemporary spatial or taxonomic scales (Linke et al. 2011, Paz-Vinas et al. 2018), and which can effectively promote multi-species management objectives (Nielsen et al. 2017; Douglas et al. 2020).

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Data Accessibility Statement

Raw FASTQ files for each individual have been uploaded to the NCBI Sequence Read Archive (BioProject ID PRJNA656098) and will be released to the public upon publication. All code is available open-source at: github.com/tkchafin/DENdriscape. Curated alignment files and inputs will be made available via the Open Science Framework (DOI: XXXXX) following acceptance.

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Figures and Tables

Table 1: Description of environmental variables used as covariates in multiple tests of riverscape genetic differentiation in Speckled Dace (*Rhinichthys osculus*) in the Colorado River Basin, where "FFR" refers to the dataset of Grill et al. (2019)

Variable	Description	Source
AET	Actual evapotranspiration (from soil water deficit); equivalent to 'soil water stress coefficient'	RiverATLAS
ARI	Aridity index	RiverATLAS
CRP	Cropland extent as percentage of reach catchment	RiverATLAS
CSI	Connectivity status index	FFR
DOF	Degree/ index of fragmentation due to artificial and/ or natural barriers	FFR
DOR	Degree/ index of anthropogenic flow regulation	FFR
FLD	Floodplain extent, as percentage of reach catchment	FFR
FOR	Forest landcover percentage in reach catchment	RiverATLAS
HFT	Standardized human footprint index	RiverATLAS
HDI	Human development index	RiverATLAS
KAR	Percentage of catchment exposed karst terrain	RiverATLAS
PAC	Percentage of reach catchment in protected areas	RiverATLAS
PET	Potential evapotranspiration predicted from WorldClim	RiverATLAS
POP	Scaled human population size in 2010	RiverATLAS
PST	Pasture extent as percentage of reach catchment	RiverATLAS
RDD	Road crossing density	FFR
RIV	River volume along reach segment	RiverATLAS
REV	Reservoir volume in watershed upstream of reach	RiverATLAS
RUN	Annual average land surface discharge and runoff	RiverATLAS
SGR	Stream gradient; ratio of elevation differential in reach and reach length	RiverATLAS
SLP	Average slope degree in catchment	RiverATLAS
SOC	Soil organic carbon content	HydroATLAS
TMP	Mean annual temperature	RiverATLAS
URB	Percentage catchment area classified as urban	RiverATLAS
USE	Proportion of water removed for human use	FFR

Table 2: Relative importance of variables (=Variable) involved with multiple methods for testing environmental association versus genetic differentiation in Speckled Dace (*Rhinichthys osculus*) from the Colorado River Basin. Values for generalized linear modeling (=GLMULTI) represent the sum of Akaike weights for models fit, using edge-wise means (=RVI_{μ}), calculated from all reaches comprising a given stream segment, and of edge-wise variation coefficients (=RVI_{cv}). Results for partial redundancy analyses (=pRDA) represent R^2 values expressed as percentage (x100), from analyses of edge-wise means (=R^2_{μ}) and variation coefficients (=R^2_{cv}). Model-averaged weights (=w_{RepN}) for each variable are presented as a component of composite resistance models fitted in RESISTNET across four replicates, where Akaike weights are computed using a maximum-likelihood population effects mixed model. Variables not meeting a RVI threshold of 0.8 (GLMULTI and RESISTNET) or not chosen using a forward-selection procedure (pRDA) are represented with a hyphen (-).

	GLMULTI		pRDA		RESISTNET			
Variable	RVI_{μ}	RVI_{cv}	R^2_{μ} (x100)	R^2_{cv} (x100)	w_{Rep1}	w_{Rep2}	w_{Rep3}	w_{Rep4}
AET	-	-	3.9%	-	0.94	-	0.55	-
ARI	-	0.990	2.9%	-	0.38	0.63	0.30	0.56
CRP	-	-	-	-	0.60	0.65	0.72	0.45
CSI	0.992	-	-	2.2%	-	-	0.51	0.49
DOF	0.971	-	1.5%	-	0.32	-	-	-
DOR	-	0.998	1.7%	-	0.53	0.63	0.60	0.59
FOR	-	-	-	-	-	-	-	-
FLD	-	0.999	-	-	-	-	-	-
HDI	0.999	-	-	-	-	-	-	-
HFT	-	-	-	-	0.62	0.58	0.93	0.56
KAR	-	-	-	-	-	-	-	-
LENGTH	-	-	-	-	-	-	-	-
PAC	0.992	-	-	-	0.70	0.25	0.35	0.24
PET	-	-	-	4.3%	0.66	0.55	0.79	0.53
POP	0.993	-	-	-	0.35	-	-	0.15
PST	-	-	-	-	-	-	-	0.08
RDD	-	-	-	-	0.42	-	-	-
REV	0.992	0.930	-	-	-	-	-	-
RIV	-	-	-	-	-	-	-	-
RUN	-	-	-	-	-	-	-	-
SED	-	-	-	-	0.45	0.75	0.77	0.75
SGR	-	-	-	-	-	0.10	0.06	-
SLP	-	-	-	1.8%	0.36	-	-	0.29
SOC	-	0.999	-	3.3%	0.28	0.41	-	0.40
TMP	0.990	0.956	-	-	-	-	-	-
URB	-	-	-	-	-	0.16	0.08	0.24
USE	0.999	0.830	2.2%	-	0.72	0.51	0.43	0.52

Figure 1: Workflows summarized for DISTNET and RESISTNET. DISTNET reduces an input shapefile to a minimal graph, using Dijkstra shortest paths between points, and merging contiguous reaches to form edges. Input genetic distances are 'fitted' to the graph using a least-squares procedure, and results are annotated against the original geometries. RESISTNET optimizes resistance models using a genetic algorithm, where a population of random models is 'evolved' given a fitness function (e.g., AIC), which is computed by comparing the effective resistances computed on the graph for each model (using CIRCUITSCAPE) against either an input pairwise genetic distance matrix, or edge-wise fitted distances from DISTNET . For each generation, models are mutated (=random parameter changes), recombined (=swapping parameter sets), selected (=included/ excluded on the basis of a fitness function), and used to generate offspring for the succeeding generation. Final results are used to compute an effective resistance network and relative variable importance values using model-averaging.

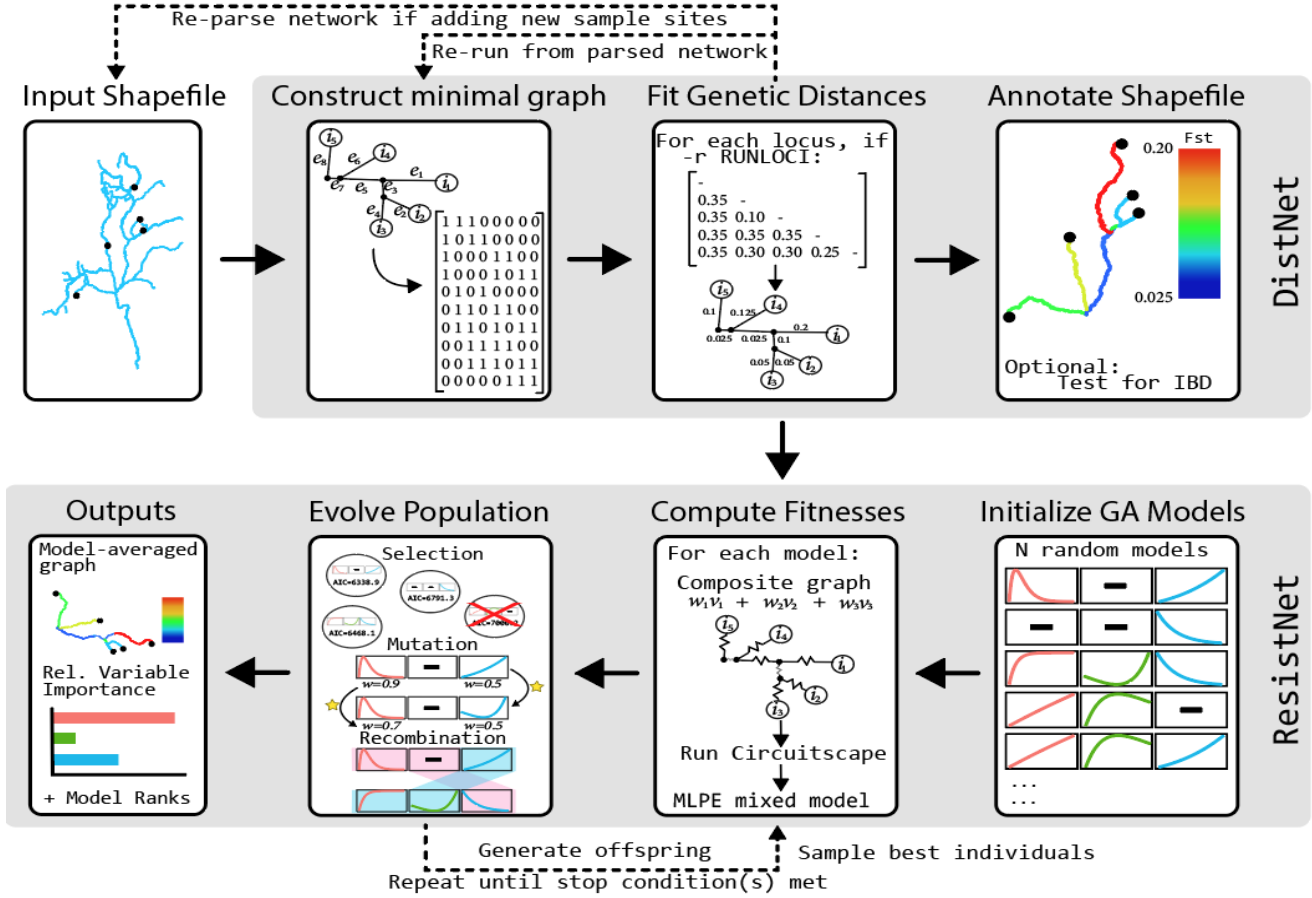


Figure 2: Distribution of *N*=78 Speckled Dace (*Rhinichthys osculus*) sample localities in the Colorado River Basin, as divided into five sub-basins: Virgin River; Grand Canyon; Little Colorado River (=Little CR); Upper Colorado River Basin (=Upper CR); and Gila+Bill Williams rivers (=Gila). Sample sites color-coded per resident sub-basin.

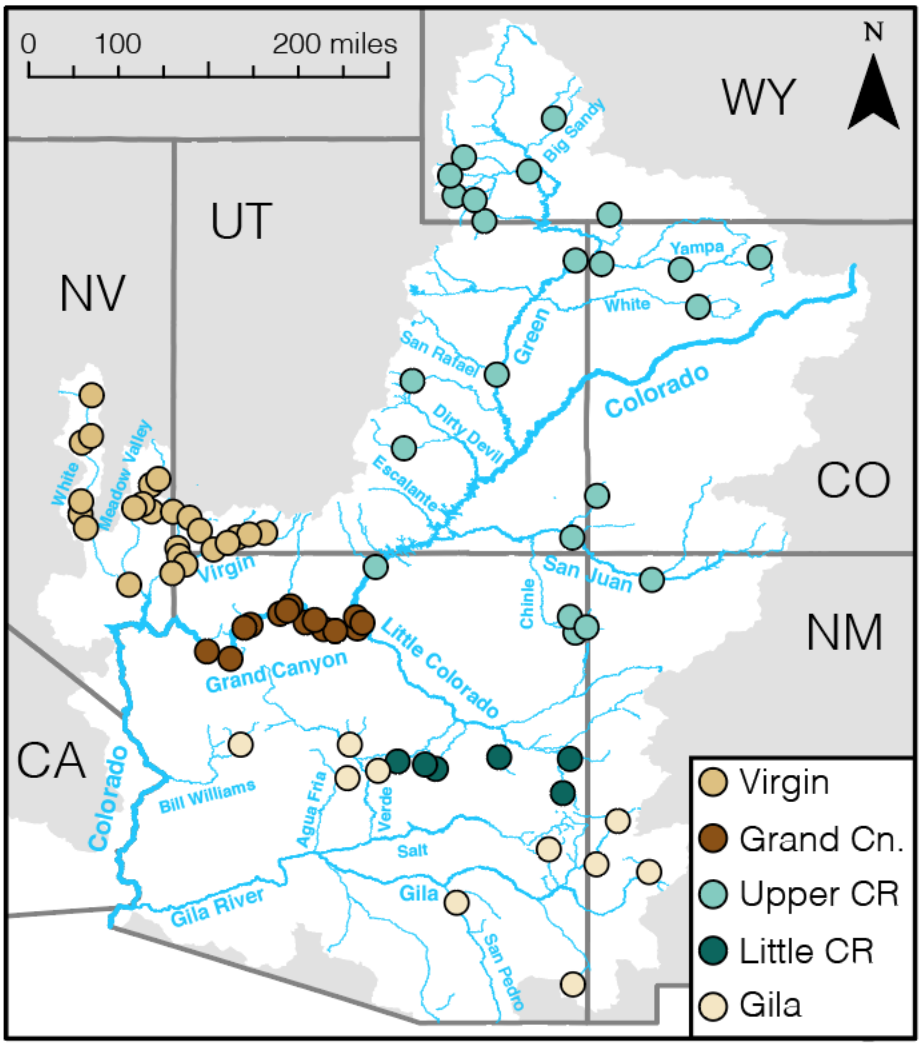


Figure 3: Population structure among Colorado River Basin (CRB) Speckled Dace populations. Upper Colorado/ Green River aggregates (*K*=6) represent: Paria River (PAR), Chinle Wash (CYC-WHY), San Juan+San Rafael+Fremont rivers (DOL-FER), Lower-Mid Green River (DES-BIG), Smiths Fork (SMF), and Vermillion Creek (NVC). The Lower CRB, represented by the Gila+Bill Williams River drainages (*K*=8) include: the Bill Williams River (FRA), Verde River (FOS-SPG), Agua Fria (SYC), San Pedro (ARU), San Simon (NCC), Eagle Creek (EAG), San Francisco River (GLN-TUL), and Upper Gila River (WFG). The Little Colorado River (*K*=4) encompasses East Clear Creek (WIL-ECC), Chevelon Creek (CHE), Silver Creek (SIC), and the East Fork (WEN-EFL). Grand Canyon (*K*=5): distributed linearly along the length of the canyon, with the exception of Havasu Creek (HAV). Virgin River (*K*=7): upper Virgin River (NTH-NFV), Mainstem Virgin River (LAV-WFA; BDW-VSC), Beaver Dam Wash (BDM-CCN), Meadow Valley Wash (BBL-MVW), Moapa River (MOA), Pahranagat (PAH-VEL), and White River (ISP-CAM).

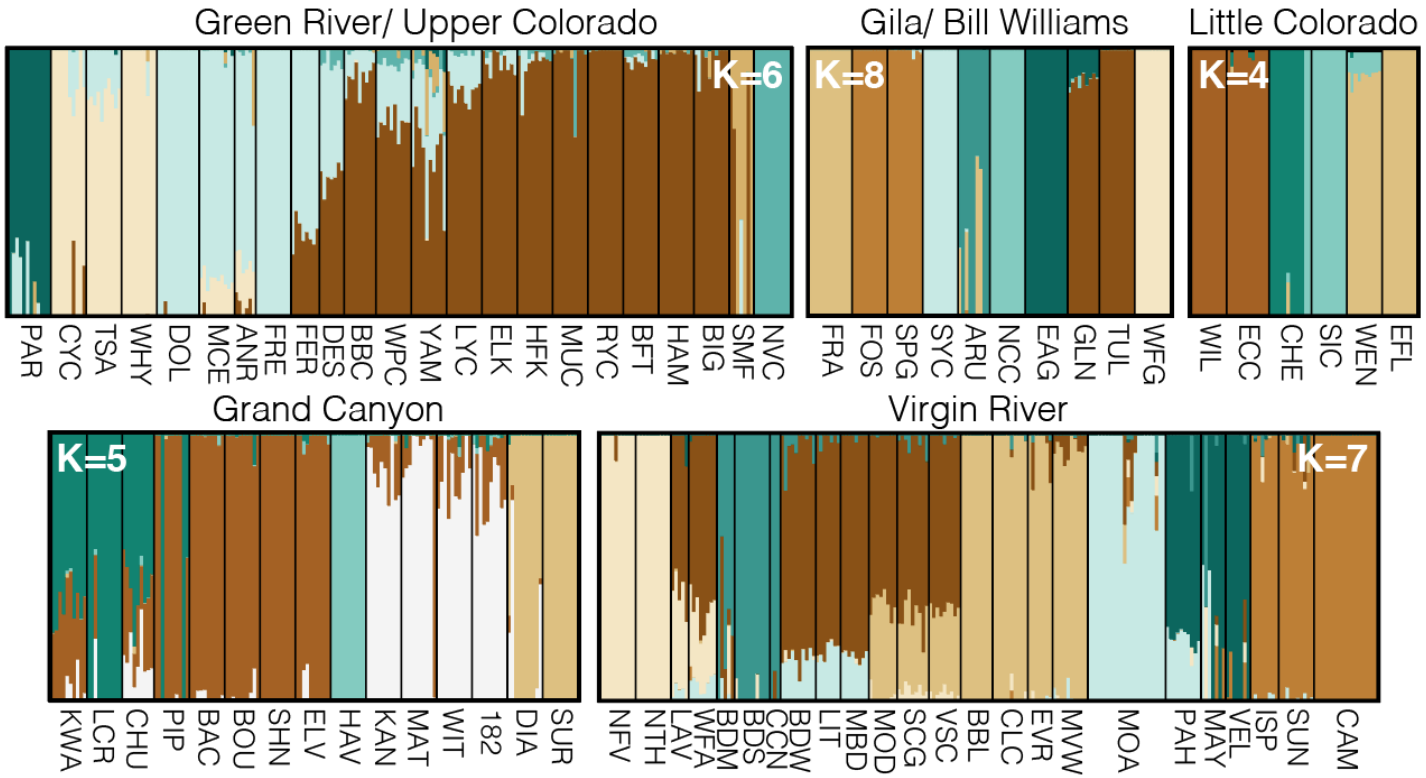


Figure 4: Composite plot of DISTNET results for N=78 Speckled Dace (*Rhinichthys osculus*) sampling localities distributed throughout the Colorado River Basin. (A) Linearized F_{ST} (F_{ST}/1-F_{ST}) computed globally across loci, fitted to stream edges; (B) Mean fitted F_{ST} estimates computed across 13,218 SNP loci; (C) Standard deviation (Std. Dev.) of per-locus fitted F_{ST} estimates; (D) Histogram of 'snap' distances between true sampling localities and nearest river network node; (E) Globally computed, linearized F_{ST} *versus* those predicted from DISTNET - fitted values (linear regression: adjusted R^2=0.77, p<1.0e^{-16}); (F) Pairwise stream distances among sites versus pairwise linearized F_{ST} (Mantel r=0.47, p<0.001).

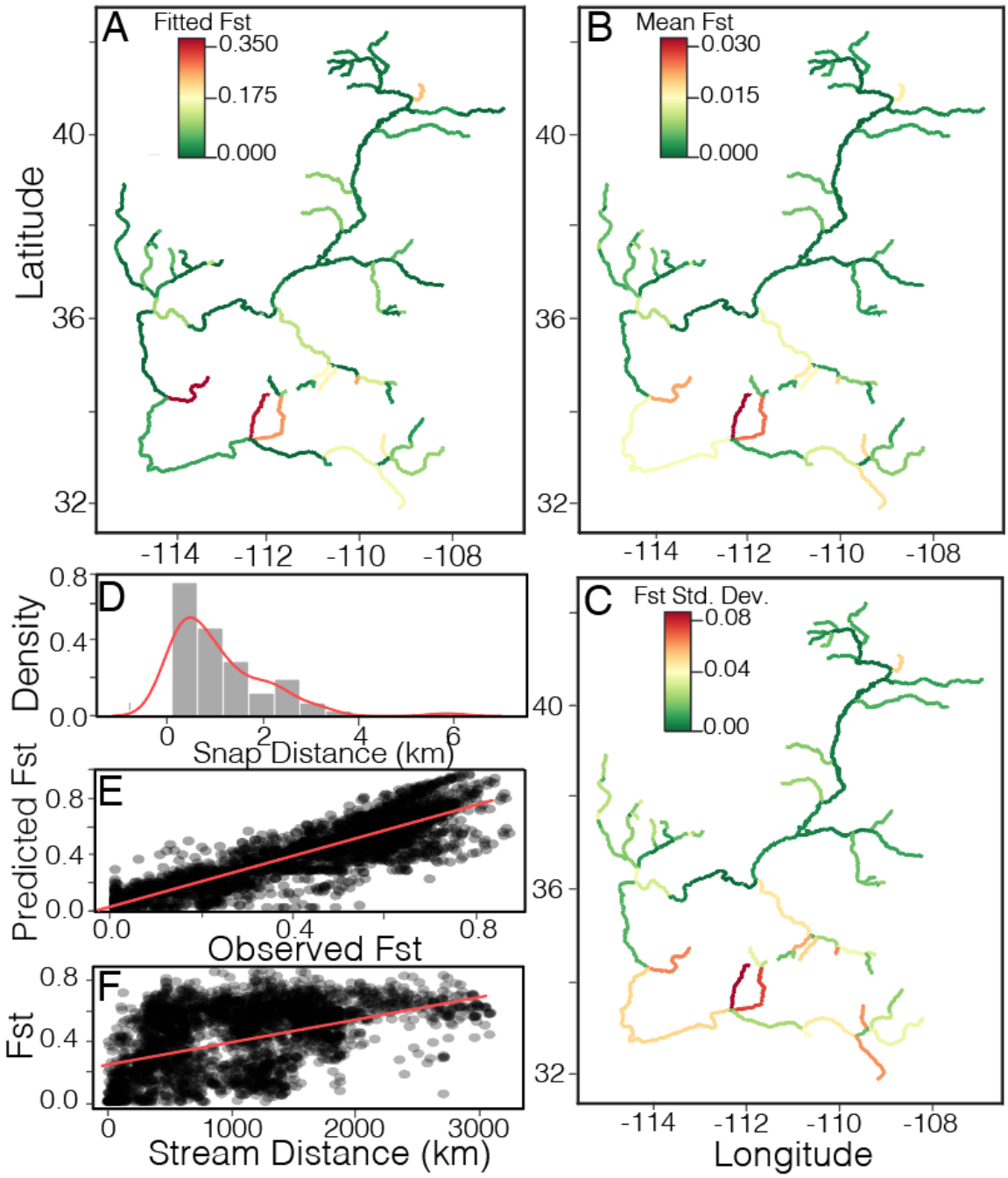


Figure 5: Modelling association of 27 environmental values and stream length with fitted-F_{ST} values for 113 contiguous stream segments (=network edges) in the Colorado River Basin, as inferred using N=78 sampling localities of Speckled Dace (*Rhinichthys osculus*) and 13,218 SNP loci and least-squares optimization in DISTNET. (A) model-averaged relative variable importance (RVI) for generalized linear models (GLMs) fit using mean F_{ST} across loci per edge (RVI_{μ}) and using variation coefficients (RVI_{cv}), with a RVI threshold of 0.8 (red line); (B-C) Partial redundancy analyses (pRDA controlled for stream length) of locus-wise F_{ST} means across edges, scaled by loci; (D-E) pRDA of locus-wise F_{ST} means, scaled by edge; and pRDA of locus-wise F_{ST} variation coefficients, scaled by loci (F-G), and by edge (H-I). pRDA variables chosen via forward selection. Variable abbreviations (e.g., AET) per Table 1.

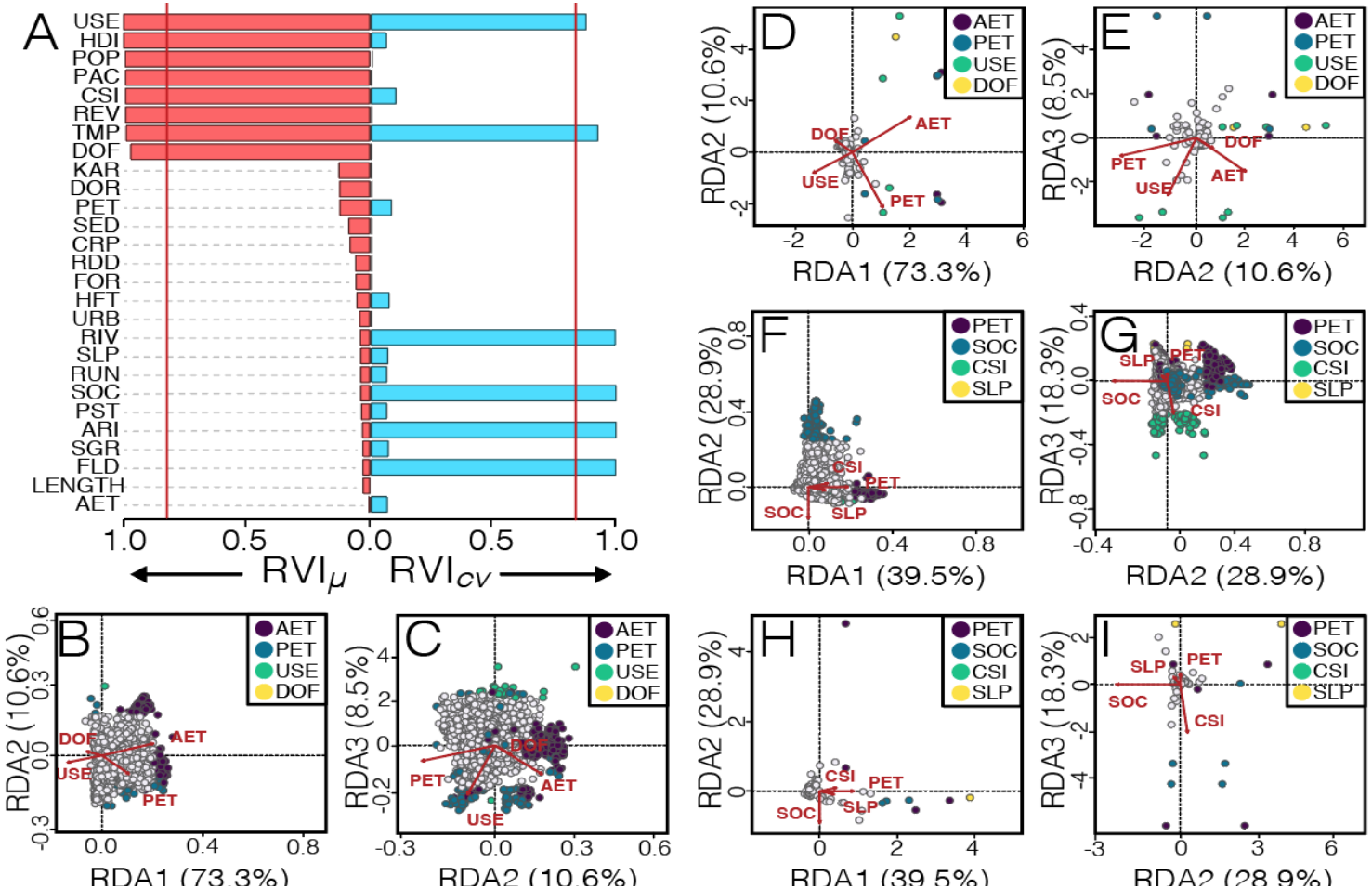
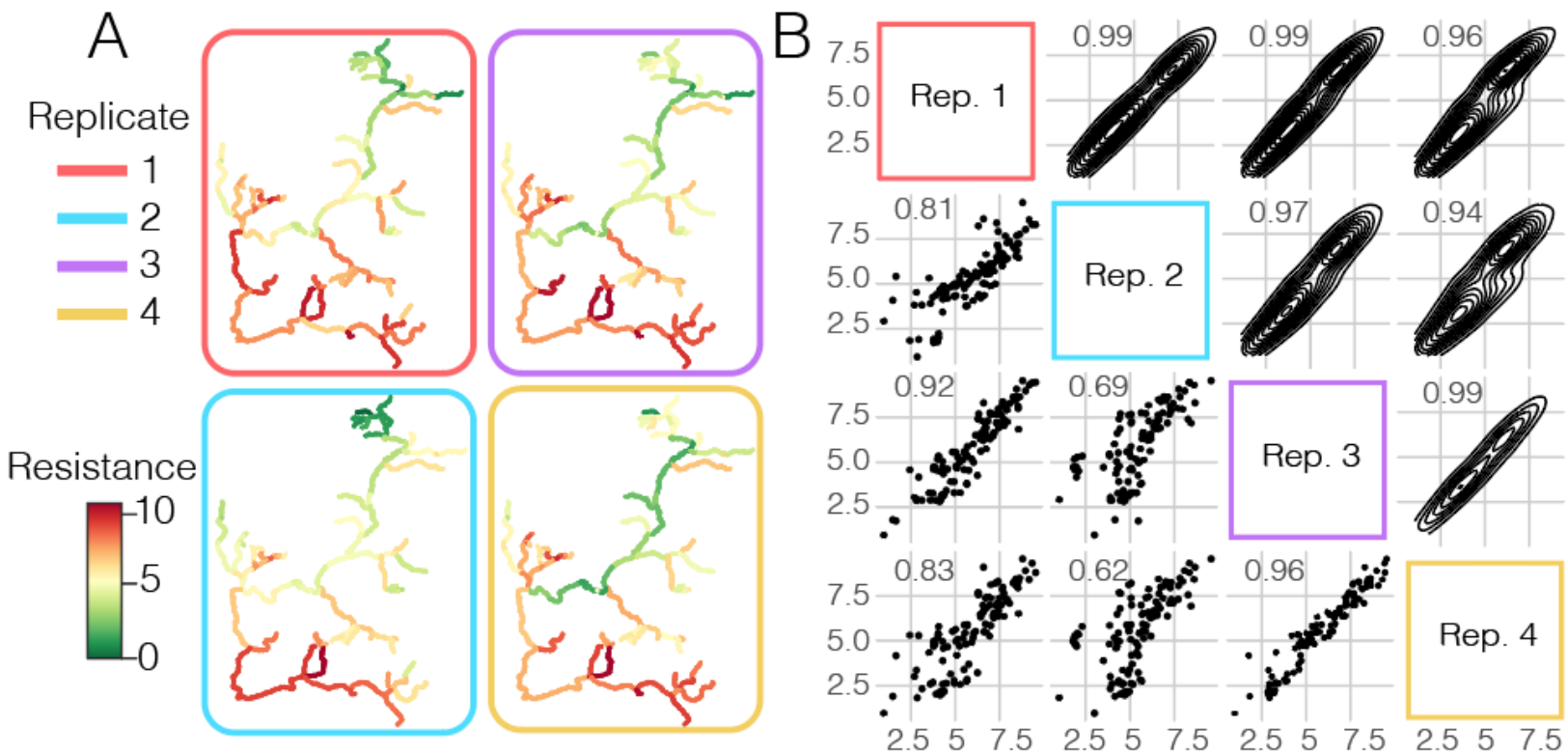


Figure 6: Composite plot of RESISTNET results across four independent replicates modelling riverscape resistance using 27 environmental variables in a river network of *N*=78 Speckled Dace (*Rhinichthys osculus*) sampling sites in the Colorado River Basin. Each replicate explored model space using a genetic algorithm with a model population size 512, with the final *N* top models selected as those with a cumulative Akaike weight of 0.95. Resistance values for each stream segment (A) represent model-averaged estimates (using Akaike weights). Replicates compared using Pearson's correlation coefficient (r) of edge-wise resistances (B; below diagonal) and Mantel test of pairwise resistances among sites (B; above diagonal).



Supplementary Material

Table S1: Locality information (=Locality) and sample sizes (=N) for *N*=78 sampling localities of Speckled Dace (*Rhinichthys osculus*) in sub-basins (=Sub-Basin) of the Colorado River Basin.

Site	Locality	Sub-basin	*N*
182	Grand Canyon RM182	Grand Canyon	10
ANR	Animas River	Upper CRB	6
ARU	Aravaipa Creek; San Pedro tributary	Lower CRB	12
BAC	Grand Canyon RM87-117	Grand Canyon	10
BBC	Big Beaver Creek – White River	Upper CRB	9
BBL	Meadow Valley Wash	Virgin	9
BDM	Beaver Dam Wash	Virgin	6
BDS	Beaver Dam Wash	Virgin	10
BDW	Mainstem Virgin River	Virgin	10
BFT	Blacks Fork	Upper CRB	10
BIG	Big Sandy River	Upper CRB	10
BOU	Grand Canyon RM87-117	Grand Canyon	10
CAM	Camp Spring – White River	Virgin	18
CCN	Beaver Dam Wash	Virgin	4
CHE	Chevelon Creek; Little Colorado trib.	Little Colorado	12
CHU	Grand Canyon RM225-249	Grand Canyon	9
CLC	Meadow Valley Wash	Virgin	10
CYC	Chinle Wash; Colorado R. tributary	Upper CRB	10
DES	Desolation Canyon; Green R. mainstem	Upper CRB	7
DIA	Grand Canyon RM225-249	Grand Canyon	10
DOL	Dolores River	Upper CRB	12
EAG	Eagle Creek	Lower CRB	12
ECC	East Clear Creek; Little Colorado trib	Little Colorado	12
EFL	East Fork, Little Colorado River	Little Colorado	10
ELK	Elk River (Yampa River Tributary)	Upper CRB	10
ELV	Grand Canyon RM87-117	Grand Canyon	10
EVR	Meadow Valley Wash	Virgin	7
FER	Ferron Creek – San Rafael River	Upper CRB	8
FOS	Fossil Creek; Verde River tributary	Lower CRB	10
FRA	Francis Creek; Bill Williams R. tributary	Lower CRB	12
FRE	Fremont River; Green River tributary	Upper CRB	10
GLN	San Francisco River at Glenwood	Lower CRB	9
HAM	Ham's Fork; Green River	Upper CRB	10
HAV	Havasu Creek, Grand Canyon	Grand Canyon	10
HFK	Ham's Fork; Green River	Upper CRB	10
ISP	Indian Springs; White River; Virgin-Muddy tributary	Virgin	8
KAN	Kanab Creek; Grand Canyon	Grand Canyon	10
KWA	Grand Canyon RM225-249	Grand Canyon	10
LAV	Mainstem Virgin River	Virgin	5

LCR	Little Colorado River confluence	Grand Canyon	10
LIT	Mainstem Virgin River	Virgin	7
LYC	Little Yampa Canyon; Yampa River	Upper CRB	10
MAT	Grand Canyon RM143-189	Grand Canyon	10
MAY	Maynard Spring – Pahranagat Valley	Virgin	7
MBD	Mainstem Virgin River	Virgin	8
MCE	McElmo Creek – San Juan River	Upper CRB	10
MOA	Moapa River; Virgin-Muddy tributary	Virgin	22
MOD	Mainstem Virgin River	Virgin	8
MVW	Meadow Valley Wash	Virgin	10
MUC	Muddy Creek – Blacks Fork River	Upper CRB	10
NCC	San Simon	Lower CRB	10
NFV	Upper Virgin River	Virgin	10
NTH	Upper Virgin River	Virgin	10
NVC	Vermillion Creek	Upper CRB	10
PAH	Pahranagat River; Virgin-Muddy basin	Virgin	10
PAR	Paria River	Upper CRB	12
PIP	Grand Canyon RM87-117	Grand Canyon	10
RYC	Ryckman Creek upstream of Hill Creek Confluence	Upper CRB	10
SCG	Mainstem Virgin River	Virgin	9
SIC	Silver Creek; Little Colorado tributary	Little Colorado	10
SHN	Shinumo Creek; Colorado R. tributary	Grand Canyon	10
SMF	Smith's Fork; Green River	Upper CRB	7
SPG	Spring Creek; Verde River tributary	Lower CRB	10
SUN	Sunnyside Creek; White River; Virgin-Muddy	Virgin	10
SUR	Grand Canyon RM225-249	Grand Canyon	10
SYC	Sycamore Creek; Agua Fria tributary	Lower CRB	10
TSA	Chinle Wash; Colorado R. tributary	Upper CRB	10
TUL	Tularosa Creek – San Francisco River	Lower CRB	10
VEL	Burn's Ranch – Pahranagat Valley	Virgin	7
VSC	Mainstem Virgin River	Virgin	10
WEN	East Fork, Little Colorado R.	Little Colorado	10
WFA	Mainstem Virgin River	Virgin	8
WFG	West Fork of the Gila River at cliff dwellings	Lower CRB	10
WIL	East Clear Creek; Little Colorado trib	Little Colorado	10
WIT	Grand Canyon RM143-189	Grand Canyon	10
WPC	Island Park below Whirlpool Canyon; Yampa River	Upper CRB	10
WHY	Chinle Wash; Colorado R. tributary	Upper CRB	10
YAM	Yampa River (Green R. tributary)	Upper CRB	10

Table S2: Outlier SNPs/ edges in pRDA analysis of means (μ) and variation coefficients (c_v).

Variable	*N* SNPs (%)	*N* Edges (%)
c_v – CSI	105 (30.1)	-
c_v – PET	112 (32.1)	3 (42.9)
c_v – SLP	2 (0.6)	1 (14.3)
c_v – SOC	130 (37.2)	3 (42.9)
μ – AET	158 (71.5)	2 (22.2)
μ – DOF	-	1 (11.1)
μ – PET	11 (5.0)	4 (44.4)
μ – USE	52 (23.5)	2 (22.2)

Table S3: Fitness metrics for the 'best' model selected in each of four independent RESISTNET replicate runs. Values represent: Log-likelihood (=*loglik*), *AIC* values, marginal R^2 of MLPE mixed model fit (=R^2_m), and change in likelihood as compared to the null model (=ΔAIC_{null}).

Replicate	*loglik*	*AIC*	R^2_m	ΔAIC_{null}
1	-3087.17	6182.35	0.337	1151.52
2	-2991.06	5990.13	0.343	1201.49
3	-3086.74	6181.49	0.296	1006.52
4	-3053.56	6115.12	0.332	1141.73

Figure S1: Shapes of transformations used in RESISTNET at several shape parameter values (1,3 ,5, 10, 20, 50, 100). Transformed values maintain the scale of the input data (with a maximum of 10.0 and minimum of 0.0), and approach linearity as the shape parameter increases.

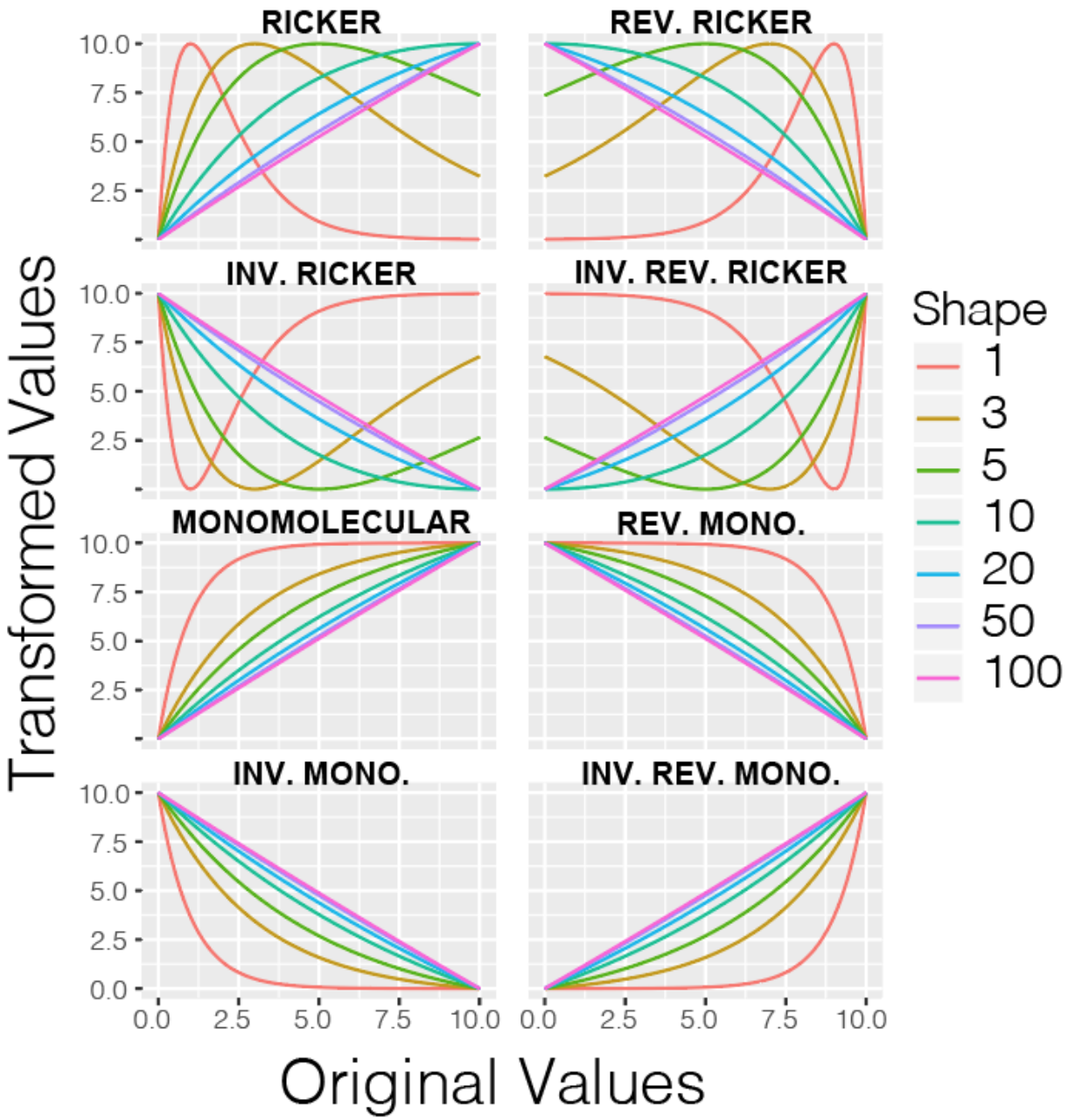


Figure S2: Graph representation of the minimized stream network for Speckled Dace (*Rhinichthys osculus*) sampling sites (N=78) in the Colorado River Basin, with sample site (blue) and junction nodes (black) represented as circles, and stream segments (edges) as connecting lines.

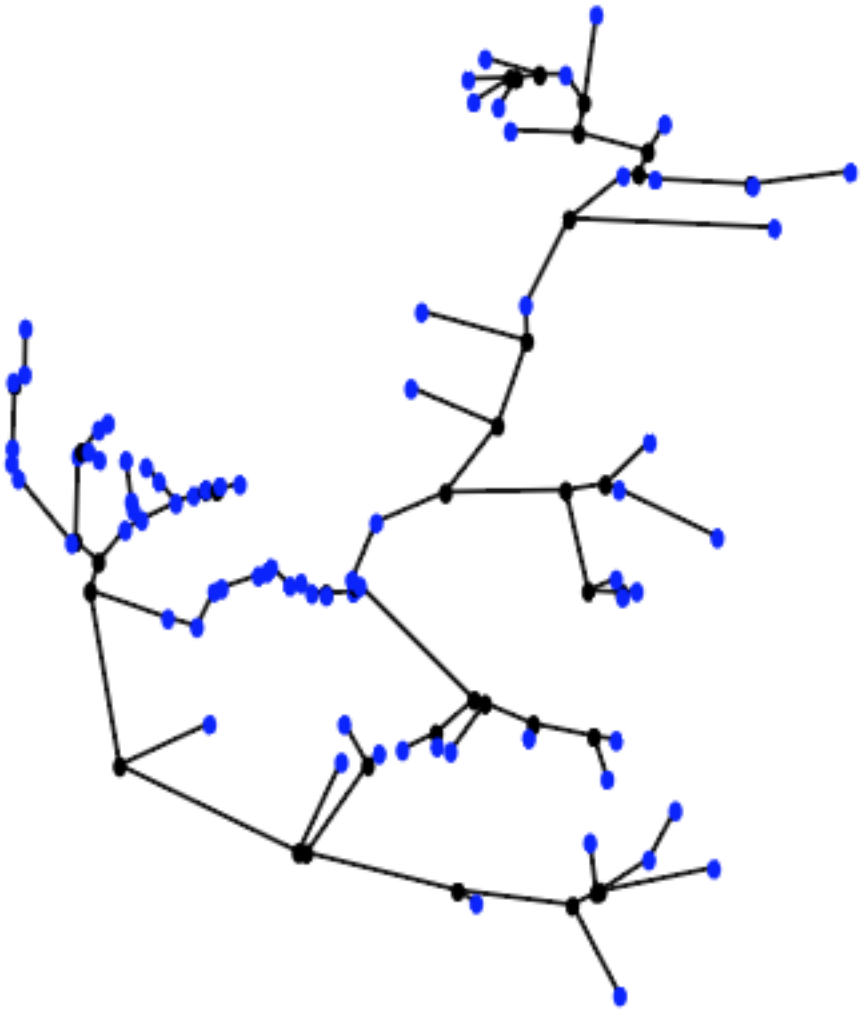


Figure S3: Benchmarking results in RESISTNET showing relationships of peak memory usage and total runtime with increasing numbers of processors. All tests used a dataset of 112 network edges (=2,248 individual contiguous stream segments) and N=78 populations. The model population was composed of 50 models examining 10 randomly selected environmental variables, with the genetic algorithm proceeding for 10 generations. All runs received the same random number seed to reduce stochasticity due to model choice. Tests were performed on a 16-core Linux computer with 256gb memory.

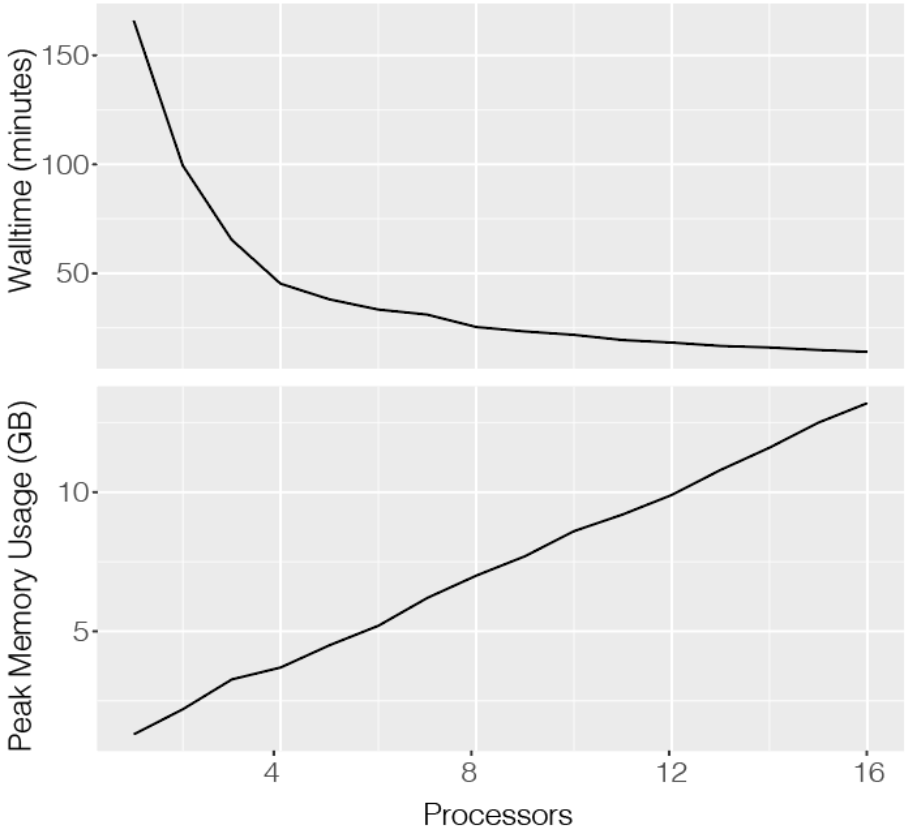


Figure S4: Benchmarking results showing increase in runtime as a function of number of generations when 16 total threads are spread as: 1) 16 parallel processes in RESISTNET, with each dedicating a single process to Circuitscape (T=16, C=1); 2) 8 RESISTNET processes, each dedicating 2 threads to Circuitscape (T=8, C=2); and 3) 4 RESISTNET processes, each dedicating 4 threads to Circuitscape (T=4, C=4). All runs evaluated an identical model population size of 50, with a fixed random number seed.

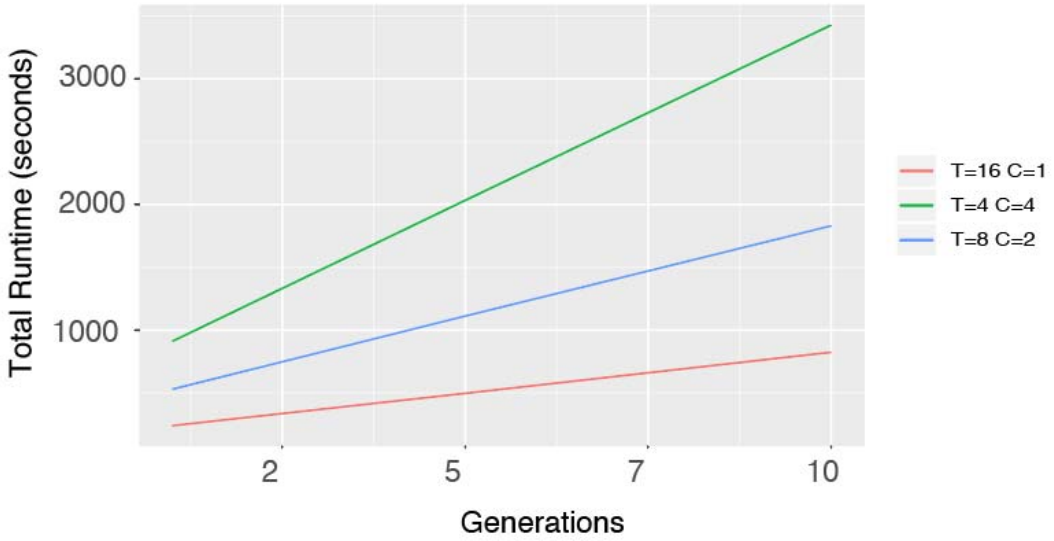


Figure S5: Fitness through time for four RESISTNET replicate runs. Solid lines show the fitness (AIC value) of the 'best' model in each generation, and the shaded region shows the range for the population of models (mean +/- standard deviation).

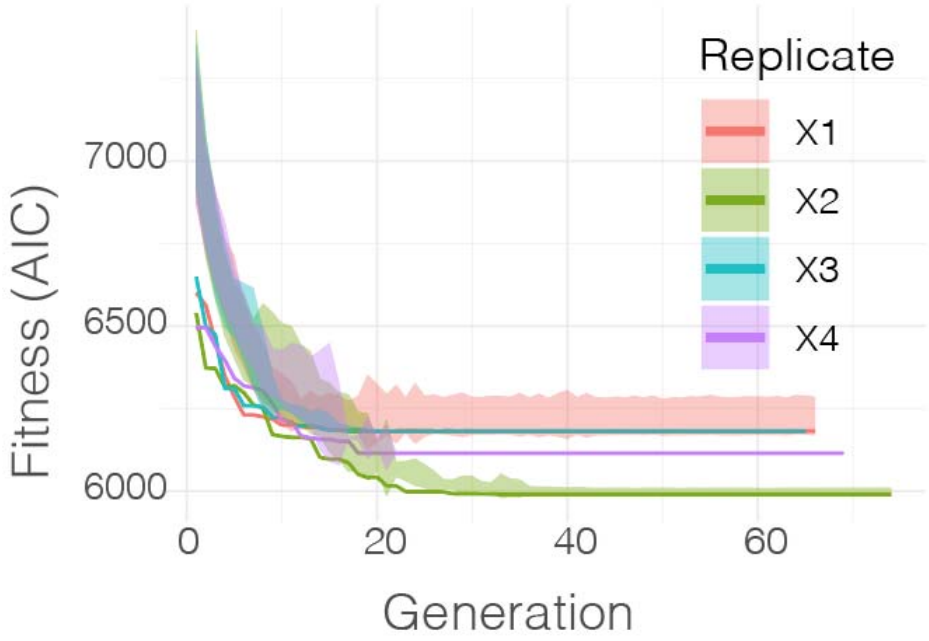
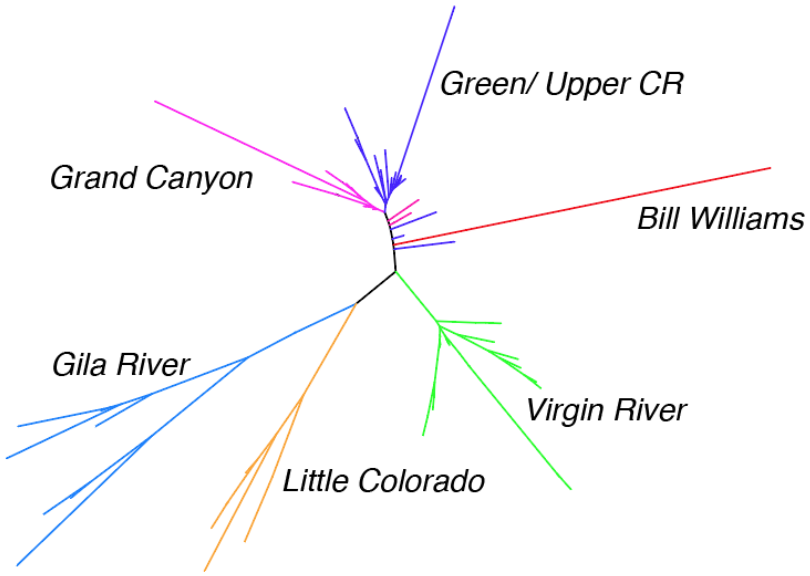



Figure S6: Unrooted species-tree generated using a polymorphism-aware model (PoMo) implemented in IQ-TREE using a GTR+G nucleotide substitution model with 4 rate categories, and a virtual population size of 19 implemented in the polymorphism model.