Spatial population structure of a widespread aquatic insect in the Colorado River Basin: Evidence for a *Hydropsyche oslari* species complex

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Abstract: Structural connectivity and dispersal ability are important constraints on functional connectivity among populations. For aquatic organisms that disperse among stream corridors, the regional structure of a river network can, thus, define the boundaries of gene flow. In this study, we used mitochondrial DNA (mtCO1 barcoding gene) to examine the genetic diversity and population structure of a caddisfly with strong dispersal capabilities, *Hydropsyche oslari* (Trichoptera:Hydropsychidae), in the topologically-diverse Colorado River Basin. We expected to find less genetic differentiation among populations of *H. oslari* within the Upper Basin, which has a dense dendritic network of perennial tributaries that allow for greater potential dispersal and gene flow, than among populations within the arid and sparse river network of the Lower Basin. We also expected to find genetic differentiation among *H. oslari* in the Upper and Lower Basins because contemporary populations are geographically distant from each other and have been separated by a >300-km-long reservoir (Lake Powell) for ½ a century. Consistent with these predictions, we found that populations of *H. oslari* within the Upper Basin had more shared haplotypes and less nucleotide diversity ($\pi = 0.001–0.008$) than *H. oslari* within the Lower Basin ($F_{ST} = 0.01$, $\pi = 0.014–0.028$). However, populations were genetically more structured in the Upper Basin ($F_{ST} = 0.47$) than in the Lower Basin ($F_{ST} = 0.01$). We also found that populations in the Upper and Lower Basin are entirely genetically differentiated ($S_{nn} = 1$), suggesting that these 2 populations were isolated thousands of years before the 1963 closure of Glen Canyon Dam and subsequent filling of Lake Powell. The most similar haplotypes among the 2 basins represent a 5.4% difference, which indicates the presence of a species complex within *H. oslari*.

Key words: Colorado River Basin, dendritic ecological networks, river networks, molecular tools, dispersal, Trichoptera, Hydropsychidae, *Hydropsyche oslari*

The geometric structure of river networks affects the distribution and dispersal of their inhabitants (Fagan 2002, Poole 2002, Campbell Grant et al. 2007, Brown and Swan 2010). In particular, the study of dendritic stream structure has contributed to theoretical advances in understanding species distribution and metapopulation connectivity (Rodriguez-Iturbe et al. 2009, Brown et al. 2011, Finn et al. 2011, Tonkin et al. 2018), and many studies have applied these ideas to freshwater organisms at the molecular scale (Miller et al. 2002, Hughes et al. 2009, Yaegashi et al. 2014). Integrating genetic approaches with river network topology provides a lens through which to evaluate the effects of structural connectivity on functional connectivity (Luque et al. 2012). In this study, we use this lens to investigate the influence of dendritic river network density on population structure and gene flow in a widespread taxon.

There are 4 general models of gene flow in drainage basins (Finn et al. 2007, Hughes et al. 2009). In river systems with high connectivity, such as dense networks of perennial streams, aquatic organisms with high dispersal ability are likely to show panmictic, uninhibited gene flow (the Widespread Gene Flow Model; Hughes et al. 2009). In isolated river segments, such as in arid landscapes where aquatic habitat is often intermittent and remote, populations are

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expected to experience highly-localized genetic drift regardless of dispersal ability (the Death Valley Model; Hughes et al. 2009). Between the 2 extremes of panmixia and total isolation, organisms are generally more likely to disperse within a watershed than among watersheds (the stream hierarchy model; Meffe and Vrijenhoek 1988), and species with overland dispersal abilities and narrow niches, such as headwater specialists, are isolated more by their narrow habitat affinities than by the structure of river networks (the headwater model; Finn et al. 2007). These models provide a useful framework for testing hypotheses of gene flow for taxa distributed across diverse riverscapes.

The topologically-diverse Colorado River Basin provides an excellent template for investigating the biological effects of river network topology on a broad scale. The watershed encompasses 640,000 km^2 of western North America and is the 7th largest watershed on the continent (Kammerer 1987). The water resources in the Colorado River Basin were divided, for political and geographical reasons, into an Upper and Lower Basin by the Colorado River Compact of 1922. Lees Ferry, 26 km downstream of Glen Canyon Dam in northern Arizona, is the division point between the 2 basins. This division has bureaucratic roots, but it is also a reasonable breakpoint for ecological studies (Fig. 1). The Upper Basin is a mesic and dendritic river network with a drainage density (total length of channel/unit area) of 0.09 km/km^2 that drains the Rocky Mountains in Colorado, the Wind River Range in Wyoming, and the Uinta Mountains in Utah. The many perennial tributaries in the Upper Basin form a dense network ranging in Strahler stream order from 1 to 7. The Lower Basin, in contrast, drains the Grand Canyon and has fewer perennial tributaries and a low drainage density of 0.02 km/km^2. The perennial tributaries to the Colorado River along its nearly 500-km course through the Grand Canyon are nearly all low-order spring-fed desert streams.

Our primary objective was to assess which model of gene flow best described the genetic diversity of a widespread aquatic insect, *Hydropsyche oslari* (Trichoptera: Hydrospychidae; Banks 1905) in the Colorado River Basin. We expected populations of *H. oslari* in the Upper Basin to align with the Widespread Gene Flow Model and be panmictic with low genetic diversity and no isolation by distance because of the interconnected habitat created by the dense network of perennial tributaries. In contrast, the mainstem river in the Lower Basin has few perennial tributaries and has a highly regulated flow regime that creates a life-history bottleneck for many aquatic insects (Kennedy et al. 2016), which results in low *H. oslari* densities. We expect these features to lead to strong isolation, small population sizes, and a high likelihood of localized genetic drift in the Lower Basin, so we expected *H. oslari* in this basin to follow the predictions of the Death Valley Model (high genetic variation, no isolation by distance). We also tested the competing hypotheses that populations in the Upper and Lower Basins would be: 1) panmictic with no differentiation among the 2 basins, 2) entirely genetically isolated, or 3) follow the stream hierarchy model and disperse more within the Upper and Lower Basins than among them. We predicted that *H. oslari* would adhere to the stream hierarchy model because the mainstem populations are separated by >600 river km. This distance, which includes Lake Powell, a 300-km-long desert-bound lentic reservoir, is a likely barrier to *H. oslari* dispersal. Finally, we tested for hierarchical groupings within and among basins to describe genetic variation among populations both individually and in distinct geographic regions. We did not sample headwater streams in this study, so we did not consider the headwater model as a hypothesis. Overall, by examining the genetic structure of a widespread aquatic insect throughout a watershed with distinctly polarized network structure, we

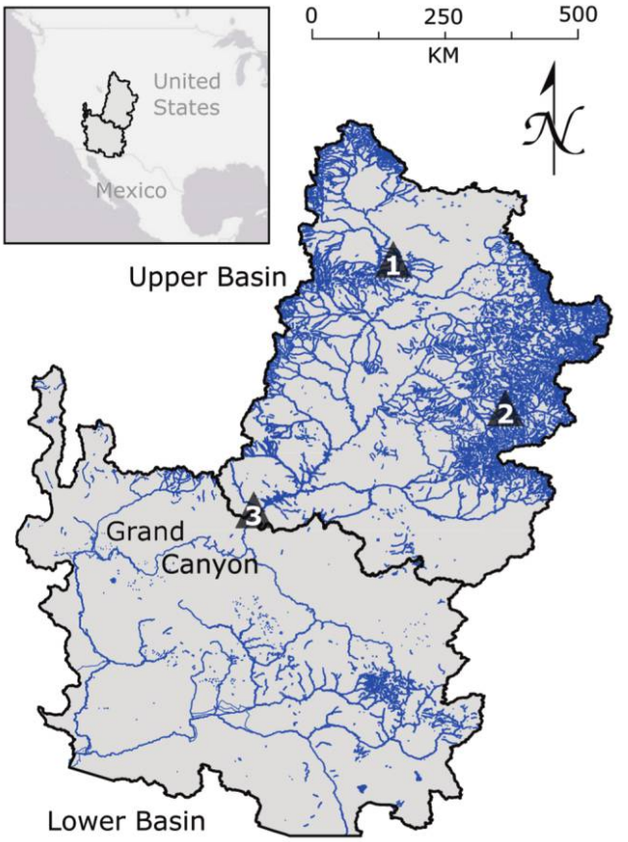

Figure 1. The mesic Upper Colorado River Basin has a dense dendritic network of perennial tributaries and a drainage density of 0.09 km/km^2. In contrast, the arid Lower Basin includes the Grand Canyon and has a network of short and sparse perennial tributaries and a drainage density of 0.02 km/km^2. Triangles depict notable dams in distinct geographic regions for our study area: Flaming Gorge Dam on the Green River (1), Crystal Dam on the Gunnison River (2), and Glen Canyon Dam on the Colorado River (3). We used the National Hydrography Dataset (https://www.usgs.gov/core-science-systems/ngp/national-hydrographyf) to calculate drainage densities.

aimed to test the influence of structural connectivity (river network density) on functional connectivity (genetic population structure) in large rivers.

METHODS

Study organism

Hydropsyche oslari is a widespread lotic species of net-spinning caddisfly (Trichoptera:Hydropsychidae) that is distributed throughout the western United States and Canada. *Hydropsyche oslari* can occur in small spring-fed streams (Flint and Herrmann 1976), mid-order streams (Alstad 1980, Schefter and Wiggins 1986), and large regulated rivers (Hauer and Stanford 1982). Their distribution and abundance are influenced by both local habitat conditions (Oswood 1976, Alstad 1980, Hauer and Stanford 1982) and intensity of intraspecific competition (Hemphill and Cooper 1983). The synonym *Ceratopsyche* was previously used by some authors (i.e., Haden et al. 1999) to describe *H. oslari*, but a review using both genetic and morphological evidence attributed this nomenclature to taxonomic inflation and reinstated the genus *Hydropsyche* for the taxon (Geraci et al. 2010).

Caddisflies (Trichoptera) are frequently included in monitoring programs as bioindicators because they are sensitive to perturbations in water quality and environmental conditions (Dohet 2002) and are important to stream food webs and ecological processes (Wallace and Merrit 1980). Most species of caddisflies spend their egg, larval, and pupal life stages underwater, only emerging from the water's surface as winged adults to mate. Post-mating, adult female caddisflies return to the water and cement eggs to submerged objects and vegetation (Ross 1944). Caddisfly dispersal varies with life stage, morphology, species, sex, and behavior as well as with environmental variables, such as flow, geomorphology, and wind (Collier and Smith 1997). Juvenile caddisflies primarily disperse downstream with river current, but winged adults can fly up, down, and perpendicular to rivers (Svensson 1974). Adults can fly as far as 650 m perpendicular to streams (Muehlbauer et al. 2014), but adult dispersal is probably most concentrated parallel to stream corridors (Peterson et al. 2004, Yaegashi et al. 2014). Mark-recapture studies along the Yagi River in Japan found that *Stenopsyche* fly primarily upstream and individual females can fly up to 12 km in a life cycle (Nishimura 1967, 1981).

In the large rivers of the Colorado River Basin, *H. oslari* occurs in the cold tailwaters of Fontenelle and Flaming Gorge Dams on the Green River and below Crystal Dam on the Gunnison River. It is also found in the less regulated lower Yampa River and in the Colorado River in the western Grand Canyon (Metcalfe 2018). Mean temperature (11.2 ± 1.9°C SD) and temperature range (>20°C seasonally) are important determinants of *H. oslari* habitat in the Colorado River Basin (Metcalfe 2018). In a 2015 to 2016 survey that deployed and analyzed 2194 light trap samples, *H. oslari* was not found in the lower Green River or in the Colorado and San Juan Rivers upstream of Lake Powell. Along the Colorado River in the Lower Basin downstream of Lake Powell, adult *H. oslari* have been collected only in low densities and close to tributaries. This distribution suggests that these individuals are from a tributary and that contemporary conditions in the Colorado River in the Grand Canyon are largely inhospitable to *H. oslari*. Indeed, fluctuations in river stage height (hydropeaking) cause mass mortality of insect eggs laid along the river's edge (Kennedy et al. 2016).

Study area

The Upper Colorado River Basin is a large, heavily-regulated and diverse system. Our uppermost sampling segment, Flaming Gorge, is downstream of Flaming Gorge Dam on the Green River (Fig. 2). The Green River is a 7th-order stream and the largest tributary to the Colorado River. Downstream of Flaming Gorge, the Green River enters a low-gradient valley (Brown's Park) and is braided and shallow for 40 km before it enters the narrow Lodore Canyon in Dinosaur National Monument. The Yampa River is a 7th-order tributary that joins the Green River 105 km downstream of Flaming Gorge Dam in a wide valley (Echo Park) that the Green River flows through for 5 km before entering Whirlpool Canyon. The Gunnison River is a 6th-order tributary of the Colorado River. We sampled this river immediately downstream of Crystal Dam and the Aspinall Unit in Black Canyon of the Gunnison National Park.

Our sampling area in the Lower Basin was confined to a 216-km segment of the Colorado River downstream of the Little Colorado River confluence in the Grand Canyon (Fig. 2). We divided our sampling reaches in the Grand Canyon into Grand Canyon East and Grand Canyon West at the Toroweap Fault, which is ~300 km downstream of Glen Canyon Dam (Fig. 2). The Toroweap Fault is one of the most active faults in Arizona and river incision rates differ up- and downstream of the fault (Pederson et al. 2002). We predicted that volcanic flows and natural damming near this fault could have influenced the historic dispersal and genetic mixing of *H. oslari*. We did not sample on the Colorado River downstream of Lake Mead, because a series of large dams causes the lower river to be nearly contiguous lentic habitat, and *Hydropsyche* were not found there in a previous survey (Blinn and Ruiter 2009).

Sample collection

We collected *H. oslari* from 5 distinct segments of the Green, Yampa, and Gunnison Rivers in the Upper Basin and 2 distinct segments from the Lower Basin (Table 1). We defined each of these 7 segments as 1 population of *H. oslari*, though samples were collected from 16 unique sites (Fig. 2). Road access to the river segments included in this study is extremely limited, but much of the study area is navigable by boat. Hence, to sample for *H. oslari* on

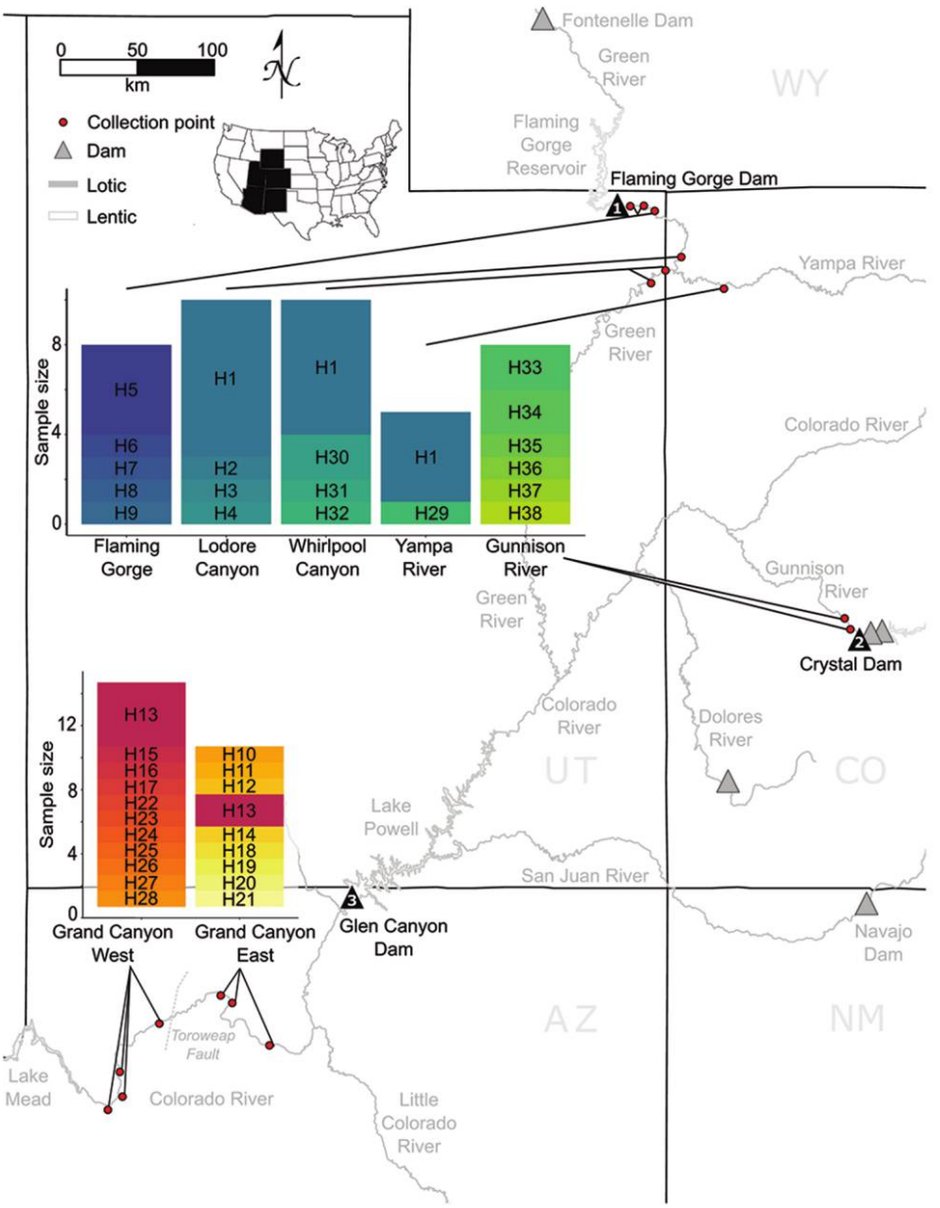

Figure 2. Map of *Hydropsyche oslari* sampling locations and haplotypes ordered by population ($n = 7$). Different colors represent unique haplotypes with shades of blue and purple depicting Upper Basin haplotypes and shades of red and yellow depicting Lower Basin haplotypes. The boundary between the Upper and Lower Basin is 26 km downstream of Glen Canyon Dam. See Table S1 for haplotypes by locality in tabular form.

a large geographic scale through remote whitewater river segments, we coordinated and trained commercial and recreational river runners to use a light trap-based collection protocol and act as citizen scientists (Kennedy et al. 2016). These citizen scientists deployed light traps most evenings (322 nights sampled in 2015, 276 in 2016) during their river expeditions and returned specimens preserved in 95% ethanol to the United States Geological Survey (USGS) facilities in Flagstaff, Arizona, for laboratory processing and preparation.

Sample processing and DNA analyses

We identified and quantified light trap sample contents in the laboratory. All *Hydropsyche* specimens were identified to species, enumerated, and stored in gasket-sealed vials of 95% ethanol. We then selected up to 14 *H. oslari* ind/pre-defined river segment for genetic analysis (Table S1). We removed *H. oslari* abdomens with equipment that we flame-sterilized between each specimen and retained the remainder of each specimen in the USGS entomological collections. We extracted DNA from the abdomen of each

Table 1. *Hydropsyche oslari* sampling locations, total specimens/sampling segment (*n*), number of haplotypes/locality, and diversity indices organized by sampling localities within the Upper (UB) and Lower (LB) Colorado River Basin.

Basin	Segment	River	State	*n*	Haplotypes	Nucleotide diversity (π)	Haplotype diversity (h)
UB				41	19	0.006	0.82
	Flaming Gorge	Green	Utah	8	5	0.004	0.79
	Lodore Canyon	Green	Colorado	10	4	0.003	0.53
	Whirlpool	Green	Utah/Colorado	10	4	0.002	0.64
	Yampa	Yampa	Colorado	5	2	0.001	0.40
	Gunnison	Gunnison	Colorado	8	6	0.008	0.93
LB				24	19	0.021	0.95
	Grand Canyon East	Colorado	Arizona	10	9	0.014	0.98
	Grand Canyon West	Colorado	Arizona	14	11	0.028	0.93

specimen with DNeasy Blood and Tissue kits (Qiagen, Valencia, California) and stored the DNA in a −20°C freezer. We used polymerase chain reaction (PCR) to amplify 612 base pairs of the mtCO1 barcoding gene. The mtCO1 gene is a mitochondrial protein coding gene, so it is maternally derived and has a high mutation rate, which makes it useful for detecting intraspecific nucleotide variation (Papadopoulou et al. 2010). We used forward primer LCO1490 (GGTCAACAAATCATAAAGATATTGG) and reverse primer Nancy (CCCGGTAAAATTAAAATATAAACTTC), a primer pair adapted for Trichoptera from Lepidopteran protocols (McCullagh et al. 2015).

We sent DNA extracts to the University of Arizona's Genomic and Technology Core Facility for PCR and sequencing. Conditions for PCR were as follows: an initial denaturation at 95°C for 5 min, 45 cycles of 95°C for 30 s, 46°C for 1 min, and 72°C for 1 min, followed by 72°C for 4 min, 4°C for 10 min, and then a hold temperature of 10°C. PCR products were purified with an ExcelaPure™ 96-well PCR purification kit (Edge Biosystems, San Jose, California), estimates of sequence amounts were quantitated with a Quant-iT™ PicoGreen™ dsDNA assay kit (Thermo Fisher Scientific, Waltham, Massachusetts), and then directly sequenced with a BigDye™ Terminator v3.a cycle sequencing kit (Thermo Fisher Scientific). Post-PCR cleanup, quantitation, and sequencing were all done according to manufacturer's protocols.

We conducted initial base calls and contig sequence assembly in Geneious (version 11.0.2, Auckland, New Zealand), a software that quantifies the concentration of each nucleotide against sequence positions and provides chromatograms to improve base call quality. We aligned nucleotide sequences by eye in MEGA (version 7.0.26; University Park, Pennsylvania) and inspected each single nucleotide polymorphism for quality. We used PARTITONFINDER (version 2.1.1; Canberra, Australia) to determine data partitions and models of evolution for each partition with greedy searches. Automated data partitioning creates and evaluates alternative models of genetic data arrangements to choose optimal data blocks and evolutionary models that are uniquely specified to each genetic dataset (Lanfear et al. 2017). Data partitioning is the first step in tree-building because partitions and their evolutionary models need to be defined a priori. We used Akaike's Information Criterion for small sample sizes to identify best models.

We constructed gene trees with Bayesian and maximum likelihood analyses done through the online CIPRES portal with MrBayes and RAXML–HPC2 Workflow (versions 3.2.6 and 8.2.10, respectively; Extreme Science and Engineering Discovery Environment, Champaign, Illinois). We used the general time-reversible model (GTR + I) for tree building because it was the optimal model selected during data partitioning. For the Bayesian analysis, we ran 2 independent 4-chain runs for 10 million generations with a 25% burn in and sampled every 1000 generations with optimal partitions. For support values, we calculated posterior probabilities for the Bayesian analyses and calculated thorough bootstrap values with 1000 iterations for the maximum likelihood analysis.

We used DNA polymorphism analysis, DNA divergence among populations analysis, and polymorphism and divergence analysis in DnaSP 6 (http://www.ub.edu/dnasp/) to compute multiple standard measures of genetic diversity within and among populations. We calculated nucleotide diversity (π), which describes the mean number of differences/site between any 2 sequences (equation 10.5 in Nei 1987). We calculated haplotype diversity (h), which describes the probability that 2 randomly-chosen haplotypes are different and ranges from 0 (no difference) to 1 (complete difference) (equation 8.4 in Nei 1987). Additionally, we calculated pairwise fixation indices (F_{ST}) (equation 3 in Hudson et al. 1992). F_{ST} describes differentiation among populations that arises from genetic structure, so values can theoretically range from 0 to 1, and values closer to 0

indicate more interbreeding. We also calculated genetic differentiation according to the nearest-neighbor statistic (S_{nn}) described in Hudson (2000). Unlike F_{ST}, S_{nn} measures the frequency of similar genetic sequences (nearest neighbors) that occur in the same geographic vicinity. If populations are similar to each other, nearest neighbors will be found throughout different populations. Therefore, high values of S_{nn} (near 1) suggest significant differentiation among populations, whereas low values of S_{nn} (near 0.5) suggest panmixia. To test for isolation by distance within and among the 2 basins, we ran Mantel tests with 9999 permutations in R (v3.4.2; R Project for Statistical Computing, Vienna, Austria) using the package *ade4* (v1.7-13; Dray and Dufour 2007) to examine correlations of pairwise F_{ST} with Euclidian (as a bird flies) and riverine (as a fish swims) distances between sample sites. We calculated nucleotide divergence to test for divergence between the 2 basins (equation 5.3 in Nei 1987). Nucleotide divergence is the average proportion of nucleotide differences between groups.

We assessed genetic variance within hierarchical structures by running an analysis of molecular variance (AMOVA) in the *ade4* package in R. For the AMOVA, we compared differences within basins and among the 7 populations occupying the geomorphologically-unique sampling segments described above. Additionally, we ran a separate AMOVA that grouped these same populations into 3 distinct geographic regions: Green and Yampa Rivers, Gunnison River, and Lower Basin (Fig. 2). For each AMOVA, genetic variance was partitioned into 3 hierarchical levels: 1) among basins, 2) among populations within basins, and 3) within populations.

We used the software TCS (version 1.21; Barcelona, Spain) and web-based tool tcsBU (Porto, Portugal) to construct a haplotype network (a visual network that shows relationships and single nucleotide polymorphism differences among haplotypes). We counted stepwise differences from the resultant haplotype network. Each stepwise difference represents a single nucleotide substitution among haplotypes. For example, 1 stepwise difference would occur if the nucleotide adenine (A) was replaced by thymine (T), 2 stepwise differences represent 2 differing nucleotides among haplotypes, and so on. We calculated percent stepwise difference as the total stepwise differences divided by the total number of base pairs multiplied by 100. We plotted haplotype diversity geographically with the R packages *maps* (v3; Becker and Wilks 2018a), *mapdata* (v.2.3; Becker and Wilks 2018b), *maptools* (v0.9; Bivand et al. 2019), and *ggplot2* (v3.2.1; Wickham et al. 2020). We constructed the map of perennial tributaries of the Colorado River Basin with ArcMap™ GIS 10.3.1 (ESRI, Redlands, California) with the perennial attribute within the streams shapefiles in the National Hydrography Dataset (USGS, Reston, Virginia). We used these data to calculate drainage density as total perennial stream length divided by watershed area.

RESULTS

From the 41 *H. oslari* individuals we collected from the Upper Basin, we identified 19 unique haplotypes (5 shared haplotypes) with 27 mutations among 24 polymorphic sites. We found high total genetic diversity ($h = 0.82$, $\pi = 0.01$) and highly structured populations ($F_{ST} = 0.47$), but low genetic differentiation among nearest-neighbor populations ($S_{nn} = 0.58$). Genetic diversity varied among the different Upper Basin populations ($h = 0.40$–0.93 and $\pi = 0.001$–0.008; Table 1) as did pairwise population structure ($F_{ST} = 0$–0.69; Table 2).

From the 24 *H. oslari* individuals we collected from the Lower Basin, we identified only 1 shared haplotype among 19 unique haplotypes (Fig. 2). Among the Lower Basin haplotypes, there were a total of 63 mutations among 61 polymorphic sites. Total genetic diversity in the Lower Basin was high ($h = 0.95$, $\pi = 0.02$), but population structure ($F_{ST} = 0.01$) and genetic differentiation ($S_{nn} = 0.56$) were low. Genetic diversity was high in both the Grand Canyon East population ($h = 0.98$, $\pi = 0.014$) and the Grand Canyon West population ($h = 0.93$, $\pi = 0.028$).

Based on the AMOVA, the genetic variation among the 7 populations within the 2 basins accounted for 42.8% of

Table 2. Fixation index (F_{ST}) values across all pairwise populations of *Hydropsyche oslari* in the Colorado River Basin, where a value of 1 indicates low interbreeding and a value of 0 indicates panmixia among populations. UB = upper basin, LB = lower basin.

	Flaming Gorge	Lodore Canyon	Yampa River	Whirlpool Canyon	Gunnison River	Grand Canyon West
Flaming Gorge (UB)	–	–	–	–	–	–
Lodore Canyon (UB)	0.59	–	–	–	–	–
Yampa River (UB)	0.69	0.00	–	–	–	–
Whirlpool Canyon (UB)	0.65	0.06	0.11	–	–	–
Gunnison River (UB)	0.31	0.38	0.48	0.45	–	–
Grand Canyon West (LB)	0.76	0.78	0.79	0.78	0.73	–
Grand Canyon East (LB)	0.85	0.87	0.89	0.88	0.83	0.01

the covariance ($p = 0.001$, df = 5, $\Phi = 0.55$; Table S2), whereas variation among all populations independent of basin accounted for 34.5% of the covariance ($p = 0.001$, df = 58, $\Phi = 0.66$; Table S2). The remaining 22.8% of the variation occurred between the Upper and Lower basins without accounting for population, but this variation was not significant ($p = 0.21$, df = 1, $\Phi = 0.23$; Table S2). Regrouping populations into 3 large populations (Green and Yampa Rivers, Gunnison River, Grand Canyon) increased the amount of covariance that was explained between populations within basins (63.3% of covariance, $p = 0.001$, df = 1, $\Phi = 0.58$; Table S3) and within populations (45.7% of covariance, $p = 0.001$, df = 62, $\Phi = 0.54$; Table S3) when compared to the first AMOVA that considered 7 populations. As with the first AMOVA, grouping to only 2 basins was not significant (−9.0% of covariance, $p = 0.654$, df = 1, $\Phi = -0.09$; Table S3).

Hydropsyche oslari collected from the Upper Basin and Lower Basin were entirely genetically differentiated ($S_{nn} = 1$) and shared no haplotypes (Figs 2, 3). Colorado River Basin-wide genetic diversity was high ($h = 0.92$, $\pi = 0.04$; Table 1), and the population was highly structured ($F_{ST} = 0.79$; Table 2). Nucleotide divergence between the Upper and Lower Basin haplogroups was 0.07%. We found 21 fixed differences and 7 shared mutations between the 2 basins (Fig. 2). There were 21 polymorphisms in the Upper Basin that were monomorphic in the Lower Basin and 57 polymorphisms in the Lower Basin that were monomorphic in the Upper Basin. These differential polymorphisms indicate that variation in haplotype diversity in individuals sampled in the 2 basins came from different loci. The shortest length of stepwise differences among individuals in the Upper and Lower Basins was 33 out of 612 total steps (pair 1: H9 and H11; pair 2: H16 and H33; Fig. 3), representing a 5.4% stepwise difference among the 2 basins.

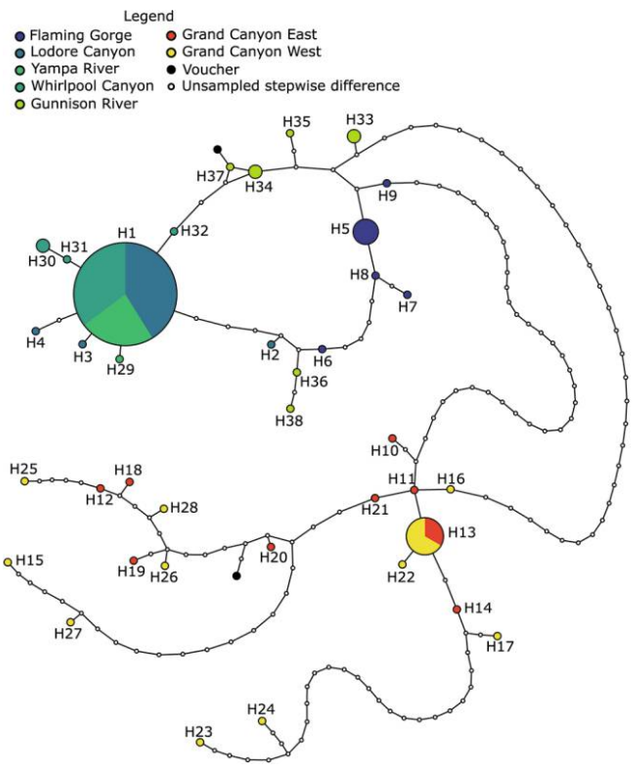

Figure 3. Diagram for *Hydropsyche oslari* showing step-wise differences among haplotypes. Colors represent different populations ($n = 7$). Large multicolored circles indicate shared haplotypes, and small white circles represent single nucleotide polymorphism differences that were not represented in our samples. We identified a total of 38 haplotypes in our analysis (Table S1), and vouchers of additional sequences are in Table S4.

Mantel tests showed that no significant pattern of isolation by distance occurred within either basin, based on either Euclidian distances (Upper: $r = -0.04$, $p = 0.55$; Lower: $r = 0.21$, $p = 0.24$) or riverine distances (Upper: $r = 0.02$, $p = 0.50$; Lower: $r = 0.17$, $p = 0.32$). However, we did find evidence for isolation by distance when we compared population structure across the entire Colorado River Basin (Fig. 4A, B) for both Euclidian ($r = 0.78$ and $p < 0.001$) and riverine ($r = 0.68$ and $p < 0.001$) distances.

Bayesian analysis resulted in a robust topology based on 15,002 trees sampled with a standard deviation of split frequencies value of 0.00075 and an average potential scale reduction factor of 1.003 (Fig. 5). The topology of the tree produced with maximum likelihood analysis had a similar topology as the Bayesian analysis (Fig. S1). Both gene trees divided individuals from the Upper and Lower Basins onto distinctly separate branches.

DISCUSSION

We found strong evidence of isolation among *H. oslari* populations in the Upper and Lower Basins. Populations in the 2 basins were genetically distinct from each other, as calculated by the nearest neighbor statistic. Genetic variance among individuals was hierarchically explained, first by differences among basins and then by differences within populations. These trends best match the predictions of the stream hierarchy model (Meffe and Vrijenhoek 1988). The division between the Upper and Lower Basins in this study is a dam rather than natural geographic features, but the Stream Hierarchy Model applies to anthropogenically-fragmented riverscapes (Hughes et al. 2009, Hopken et al. 2013) as well as natural landscapes. Lake Powell, a 300-km-long reservoir located in the southern Utah desert, is an obvious and formidable dispersal barrier to hydropsychid caddisflies that rely on lotic habitat during their juvenile life stages.

The large stepwise difference that separated the most similar *H. oslari* individuals of the Upper and Lower Basin populations suggests the isolation among the basins significantly pre-dates the 1963 construction of Glen Canyon Dam and creation of Lake Powell at this basin boundary.

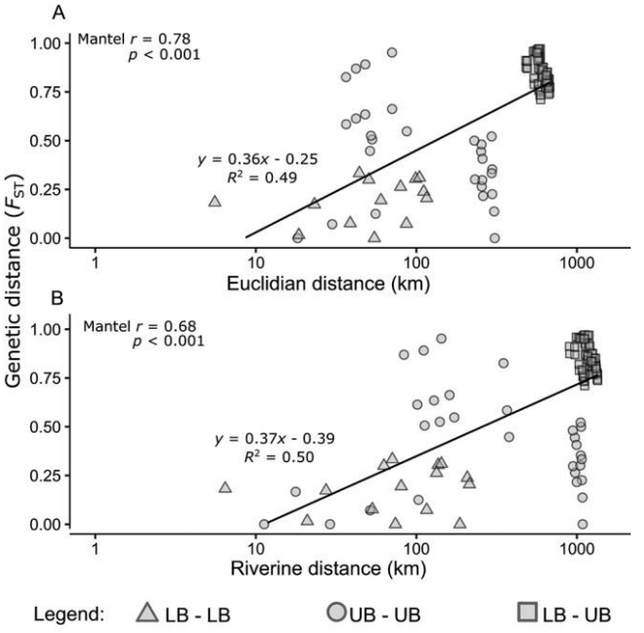

Figure 4. Relationships between F_{ST} of *Hydropsyche oslari* populations and distance in the Colorado River Basin based on both Euclidian (A) and riverine (B) distances. Regression lines indicate isolation by distance across basins, and point shapes describe geography of pairwise comparisons among the Upper (UB) and Lower (LB) Basins.

We lack sufficient data to estimate time since divergence with calibrated molecular clock methods (Gaunt and Miles 2002, Papadopoulou et al. 2010), but we used Brower's (1994) uncalibrated divergence rate of 2.3% per million years to our nucleotide divergence measure of 0.07%, which yielded an estimated time since divergence between Upper and Lower Basin populations of ~30,000 y. The accuracy of uncalibrated molecular clocks for estimating time since isolation has not been resolved (Wilke et al. 2009). However, the scale of divergence we found among basins strongly suggests that *H. oslari* in the Upper and Lower Basins were separated during the Pleistocene epoch and, therefore, long before the construction of Glen Canyon Dam. Understanding the exact cause of this ancient split will require further investigation, but increased volcanic activity and dam-building lava flows in the Colorado River Basin during the Pleistocene may have created historical dispersal barriers (Duffield et al. 2006).

The 5.4% stepwise difference between *H. oslari* in the 2 basins also suggests that these individuals represent a species complex of at least 2 genetically-distinct and spatially-segregated taxa. We define a species complex here as groups of organisms that are genetically differentiated, morphologically differentiated, or both but have not been taxonomically delineated as distinct species in a peer reviewed publication. Previous work defined species within Trichoptera at a 2% level of genetic divergence (Sweeney et al. 2011). Analysis of larvae in the Pacific Northwest has suggested that *H. oslari* may be a species complex based on morphological characteristics (Schefter and Wiggins 1986). Additionally, in a parallel *Hydropsyche* study, we observed phenotypic differentiation among adult *H. oslari*. Female *H. oslari* in the Upper Basin have wider legs (specifically, the mesothoracic tibiae and first tarsal segments) than females in the Lower Basin. Sexual dimorphism among hydropsychids in which females have wider legs than males is considered to be a swimming adaptation for deep-water egg laying (Deutsch 1985).

The habitat preferences and contemporary distributions of *H. oslari* provide further insight to the isolation of Upper and Lower Basin populations. Populations are found in river segments with relatively-cold water temperatures, such as the tailwaters below dams. *Hydropsyche oslari* do not occur in the Colorado or Green River for hundreds of km upstream of Lake Powell, where water temperatures are warmer and more variable than in tailwaters (Metcalfe 2018). Little is known about invertebrate assemblages in the Grand Canyon prior to the construction of Glen Canyon Dam (but see Woodbury et al. 1959), but it is unlikely that *H. oslari* would have colonized the warm, turbid, and hydrologically-variable conditions of the Grand Canyon pre-dam (Stevens et al. 1997). Catch rates of *H. oslari* in the Grand Canyon are greatest near tributaries, which are the most likely source populations and refugia for this taxon in the Lower Basin because hydropeaking limits population size in the mainstem Colorado River (Metcalfe 2018).

Isolation to tributaries and dispersal limitations are common explanations for the distribution of animals such as *H. oslari* that have specific habitat requirements. In Europe, genetically-distinct populations of montane limnephilid caddisflies (*Drusus discolor*) were found to be restricted to headwater streams in mountain ranges, having become isolated within glacial refugia over millions of years as suitable habitat shrank with warming climates (Pauls et al. 2006). Such isolation of taxa to headwater tributaries has been found among both strong and weak dispersing taxa (Rader et al. 2019), though it is generally predicted that organisms with high dispersal ability experience less genetic drift.

Indeed, populations of hydropsychid caddisflies have been described as panmictic across substantial distances in previous studies. For example, populations of *Hydropsyche exocellata* had very low genetic differentiation along a 200-km segment of the Loire River, the largest river in France (Guinand and Tachet 2000). Similarly, there was no spatial genetic structure in *Cheumatopsyche* sp. AV1 within or between the 3 large and arid watersheds near Sydney, Australia, that range in size from 100 to 20,000 km^2 (Baker et al. 2003). Another study, which included 97 perennial and intermittent streams in 4 basins that spanned nearly 10,000 km^2 on the Iberian Peninsula, found that *Hydropsyche siltalai* dispersed freely among basins and had no significant population structure (Múrria et al. 2010). Our total study area substantially exceeded that of these studies (the entire Colorado

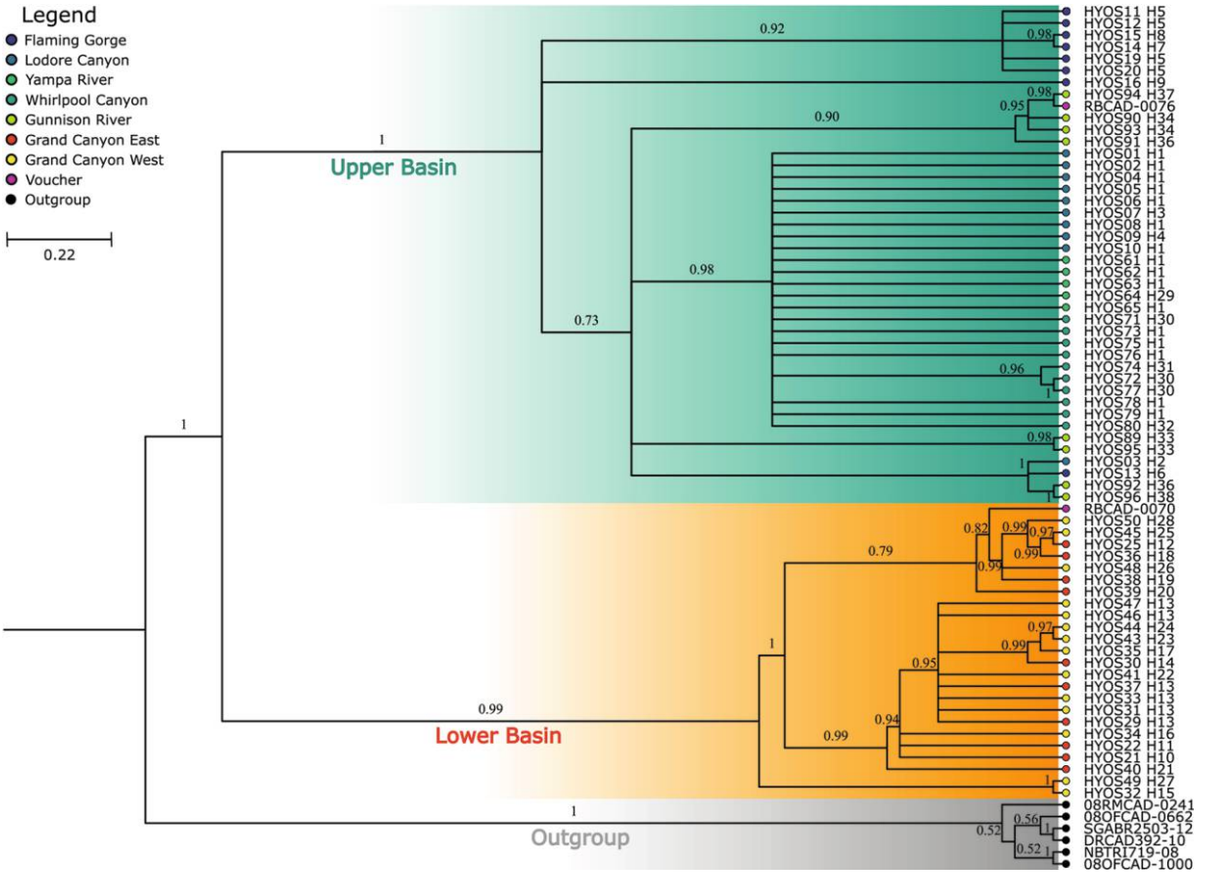

Figure 5. Bayesian gene tree output with probability values marked above branches. Blue and orange shading mark *Hydropsyche oslari* individuals collected from the Upper and Lower Basins, respectively. Gray shading marks other species of *Hydropsyche* used as the outgroup to root the tree (Table S4). The scale bar and branch lengths represent the mean number of substitutions.

River Basin is 640,000 km^2), indicating that strong-dispersing hydropsychids are only limited in instances of extreme distances between suitable habitat patches. Our AMOVA results also support this model because grouping populations over a wide geographic scale (3 populations: Green and Yampa Rivers, Gunnison River, and Lower Basin) described more of the genetic variation within each basin than did separating populations into 7 smaller groups. None of our populations were truly panmictic (no haplotype variation), but broad groupings helped explain genetic variance because some haplotypes were shared across large geographic regions.

Within the Upper Basin, *H. oslari* shared several haplotypes across populations and had far fewer stepwise differences relative to populations within the Lower Basin. Relative to other studies, however, genetic structure within the Upper Basin was high ($F_{ST} = 0.47$). For example, F_{ST} values for stone-cased caddisflies (*Tasimia palpata*) collected in southeast Queensland, Australia, ranged from 0 to 0.14 and had no significant genetic differentiation (Schultheis and Hughes 2005). Myers et al. (2001) found F_{ST} values that ranged from 0 to 1 for the little brown sedge (*Lepidostoma ojanum)* and from 0.3 to 0.6 for the silver-striped sedge (*Hesperophylax designatus*) across isolated springs along the Nevada-California border, USA. Pairing these values with data on the species' dispersal ability and varied morphology, the authors concluded that populations of the little brown sedge are genetically isolated but populations of the silver striped sedge are linked. Additionally, silver striped sedges are excellent dispersers with highly variable population structure. We cannot fully reject the widespread gene flow model in the Upper Basin despite its relatively-high F_{ST} value (0.47), because populations shared several haplotypes and had few stepwise differences among individuals. Our finding of no substantial isolation by distance within the Upper Basin combined with the high density of *H. oslari* populations previously observed in the Upper Basin (Metcalfe 2018) suggests that moderate gene flow and dispersal occurs between these populations.

Population structure within the Lower Basin indicates restricted genetic connectivity and possibly a genetic bottleneck. Haplotypes within the Lower Basin were highly differentiated (Fig. 3). We found as many as 24 stepwise differences among specimens collected within 27 km of one

another in the Grand Canyon (haplotypes H17 and H24), which is a short geographic distance relative to the large scale of our study area. The low F_{ST} value (0.01) indicates that gene flow occurs between our 2 predefined Grand Canyon populations. However, this low F_{ST} value stemmed from a single shared haplotype (H13) out of 19 haplotypes. Haplotype H13 was found in 2 individuals in the eastern population and 4 individuals in the western population, a relatively high frequency for a sample size of 24 individuals. Thus, our 2 predefined population groupings in the Grand Canyon (East and West of the Toroweap Fault) do not appear to represent a significant boundary for haplotype H13. The haplotype network for the lower basin included many rare haplotypes separated by large stepwise differences radiating from a single shared haplotype (Fig. 3). Combined with these large stepwise differences, the high nucleotide and haplotype diversity indices suggest that most haplotypes of *H. oslari* within the Lower Basin, with the exception of H13, have been isolated for long periods of time. A study of giant water bugs (*Appasus japonicus* and *A. major*) in East Asia found similar shaped haplotype networks with many rare haplotypes separated by multiple stepwise differences and attributed the shape to geohistorical isolation and a possible bottleneck (Suzuki et al. 2014). We did not find any conclusive evidence of a genetic bottleneck (Table S5), but it is one potential explanation based on the layout of the Lower Basin haplotype network.

The combination of sparse river network connectivity (limited habitat and dispersal corridors) and hydropeaking flows in the Grand Canyon probably severely limits successful oviposition events in our Lower Basin study area, leading to the high and unexpected genetic variation in addition to the lack of isolation by distance that we observed in this study. Genetic patterns similar to those we found in the Lower Basin have been described as patchy recruitment in other instances (Hughes et al. 2009). For example, in the rainforest streams of southeastern Queensland an undescribed mayfly species (*Baetis* sp.) and a tasmiid caddisfly (*Tasiagma ciliate*) were found to have irregular trends in genetic diversity, characterized by many unique haplotypes, that reflected patchy oviposition events cause by unpredictable climate and asynchronous emergence phenologies (Hughes et al. 1998). Our finding of one common haplotype (H13) in the Grand Canyon and many isolated and differentiated other haplotypes may also be a product of patchy recruitment.

Finally, our hypothesis that genetic structure in *H. oslari* is influenced by river network topology is supported by our findings of less genetic diversity and population structure in the dense network of perennial tributaries in the Upper Basin than in the arid and linear river network of the Lower Basin. Though *H. oslari* are strong dispersers, limited network connectivity in the Lower Basin is the likely cause of increased isolation and rare haplotype variation. A genetic analysis of Bluehead Suckers (*Catostomus discobolus*) in the Colorado River Basin found the same relationship between network topology and genetic diversity because resident Bluehead Sucker populations in the Grand Canyon and the topologically similar Canyon de Chelly both had lower gene flow and genetic diversity than Upper Basin populations (Hopken et al. 2013). This pattern is not limited to the Colorado River Basin. For example, the genetic diversity of Chum Salmon (*Oncorhynchus keta*) in Alaskan rivers was also lowest in river networks with the least complexity (Olsen et al. 2008).

Through examining the genetic structure of a widespread aquatic insect in a watershed with a distinctly bimodal network structure, we found that structural connectivity affects functional connectivity for populations of *H. oslari* in the Colorado River Basin. Indeed, we found such pronounced variation among the 2 basins in this study that we propose that *H. oslari* is a species complex. Regional drivers of species distribution, such as river network topology, should be considered in research that investigates dispersal and genetic diversity over large geographic expanses (Brown and Swan 2010). Further investigation of genetic variation, both within *H. oslari* and for other taxa in the Colorado River Basin, is warranted.

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LITERATURE CITED

Alstad, D. 1980. Comparative biology of the common Utah Hydropsychidae (Trichoptera). American Midland Naturalist 103:167–174.

Baker, A. M., S. A. Williams, and J. M. Hughes. 2003. Patterns of spatial genetic structuring in a hydropsychid caddisfly (*Cheumatopsyche* sp. AV1) from southeastern Australia. Molecular Ecology 12:3313–3324.

Becker, R. A., and A. R. Wilks. 2018a. *maps*: Draw geographical maps. (Available from: https://CRAN.R-project.org/package=maps)

Becker, R. A., and A. R. Wilks. 2018b. Package *mapdata*: Extra map databases. (Available from: https://cran.r-project.org/web/packages/mapdata/mapdata.pdf)

Bivand, R., N. Lewin-Kohl, E. Pebesma, E. Archer, A. Baddeley, N. Bearman, H.-J. Bibiko, S. Brey, J. Callahan, G. Carrillo, S. Dray, D. Forrest, M. Friendly, P. Giraudoux, D. Golicher, V. G. Rubio, P. Hausmann, K. O. Hufthammer, T. Jagger, K. Johnson, S. Luque, D. MacQueen, A Niccolai, O. P. Lamigueiro, E. Plunkett, T. Short, G. Snow, B. Stabler, M. Stokely, and R. Turner. 2019. *maptools*: Tools for handling spatial objects. (Available from: https://CRAN.R-project.org/package=maptools)

Blinn, D. W., and D. E. Ruiter. 2009. Caddisfly (Trichoptera) assemblages along major river drainages in Arizona. Western North American Naturalist 69:299–308.

Brower, A.V. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences 91:6491–6495.

Brown, B. L., and C. M. Swan. 2010. Dendritic network structure constrains metacommunity properties in riverine ecosystems. Journal of Animal Ecology 79:571–580.

Brown, B. L., C. M. Swan, D. A. Auerbach, E. H. Campbell Grant, N. P. Hitt, K. O. Maloney, and C. Patrick. 2011. Metacommunity theory as a multispecies, multiscale framework for studying the influence of river network structure on riverine communities and ecosystems. Journal of the North American Benthological Society 30:310–327.

Campbell G., E. H., W. H. Lowe, and W. F. Fagan. 2007. Living in the branches: Population dynamics and ecological processes in dendritic networks. Ecology Letters 10:165–175.

Collier, K. J., and B. J. Smith. 1997. Dispersal of adult caddisflies (Trichoptera) into forests alongside three New Zealand streams. Hydrobiologia 361:53–65.

Deutsch, W. G. 1985. Swimming modifications of adult female Hydropsychidae compared with other Trichoptera. Freshwater Invertebrate Biology 4:35–40.

Dohet, A. 2002. Are caddisflies an ideal group for the biological assessment of water quality in streams? Proceedings of the 10th International Symposium on Trichoptera 15:507–520.

Dray, S., and A. Dufour. 2007. The *ade4* package: Implementing the duality diagram for ecologists. Journal of Statistical Software 22:1–20. (Available from: https://www.rdocumentation.org/packages/ade4/versions/1.7-13/topics/ade4-package)

Duffield, W., N. Riggs, D. Kaufman, D. Champion, C. Fenton, S. Forman, W. McIntosh, R. Hereford, J. Plescia, and M. Ort. 2006. Multiple constraints on the age of a Pleistocene lava dam across the Little Colorado River at Grand Falls, Arizona. Geological Society of America Bulletin 118:421–429.

Fagan, W. F. 2002. Connectivity, fragmentation, and extinction risk in metapopulations. Ecology 83:3243–3249.

Finn, D. S., M. S. Blouin, and D. A. Lytle. 2007. Population genetic structure reveals terrestrial affinities for a headwater stream insect. Freshwater Biology 52:1881–1897.

Finn, D. S., N. Bonada, C. Múrria, and J. M. Hughes. 2011. Small but mighty: Headwaters are vital to stream network biodiversity at two levels of organization. Journal of the North American Benthological Society 30:963–980.

Flint Jr, O. S., and S. J. Herrmann. 1976. The description of, and environmental characterization for, a new species of *Ochrotrichia* from Colorado (Trichoptera: Hydroptilidae). Annals of the Entomological Society of America 69:894–898.

Gaunt, M. W., and M. A. Miles. 2002. An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. Molecular Biology and Evolution 19:748–761.

Geraci, C. J., X. Zhou, J. C. Morse, and K. M. Kjer. 2010. Defining the genus *Hydropsyche* (Trichoptera: Hydropsychidae) based on DNA and morphological evidence. Journal of the North American Benthological Society 29:918–933.

Guinand, B., and H. Tachet. 2000. Population structure of *Hydropsyche exocellata*. Genetic homogeneity in a zone of fragmented distribution. Comptes Rendus de l'Académie des Sciences, Series III, Sciences de la Vie 323:757–768.

Haden, A., D. W. Blinn, J. P. Shannon, and K. P. Wilson. 1999. Interference competition between the net-building caddisfly *Ceratopsyche oslari* and the amphipod *Gammarus lacustris*. Journal of Freshwater Ecology 14:277–280.

Hauer, F. R., and J. A. Stanford. 1982. Ecological responses of hydropsychid caddisflies to stream regulation. Canadian Journal of Fisheries and Aquatic Sciences 39:1235–1242.

Hemphill, N., and S. D. Cooper. 1983. The effect of physical disturbance on the relative abundances of two filter-feeding insects in a small stream. Oecologia 58:378–382.

Hopken, M. W., M. R. Douglas, and M. E. Douglas. 2013. Stream hierarchy defines riverscape genetics of a North American desert fish. Molecular Ecology 22:956–971.

Hudson, R. R. 2000. A new statistic for detecting genetic differentiation. Genetics 155:2011–2014.

Hudson, R. R., M. Slatkin, and W. P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics 132:583–589.

Hughes, J. M., S. E. Bunn, D. A. Hurwood, and C. Cleary. 1998. Dispersal and recruitment of *Tasiagma ciliata* (Trichoptera: Tasimiidae) in rainforest streams, south-eastern Australia. Freshwater Biology 39:117–127.

Hughes, J. M., D. J. Schmidt, and D. S. Finn. 2009. Genes in streams: Using DNA to understand the movement of freshwater fauna and their riverine habitat. Bioscience 59:573–583.

Kammerer, J. C. 1987. Largest rivers in the United States. United States Geological Survey. (Available from: https://pubs.usgs.gov/of/1987/ofr87-242/)

Kennedy, T. A., J. D. Muehlbauer, C. B. Yackulic, D. A. Lytle, S. W. Miller, K. L. Dibble, E. W. Kortenhoeven, A. N. Metcalfe, and C. V. Baxter. 2016. Flow management for hydropower extirpates aquatic insects, undermining river food webs. Bioscience 7:561–575.

Lanfear, R., P. B. Frandsen, A. M. Wright, T. Senfeld, and B. Calcott. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34:772–773.

Luque, S., S. Saura, and M. J. Fortin. 2012. Landscape connectivity analysis for conservation: Insights from combining new methods with ecological and genetic data. Landscape Ecology 27:153–157.

McCullagh, B., S. Wissinger, and J. Marcus. 2015. Identifying PCR primers to facilitate molecular phylogenetics in Caddisflies (Trichoptera). Zoological Systematics 40:459–469.

Meffe, G. K., and R. C. Vrijenhoek. 1988. Conservation genetics in the management of desert fishes. Conservation Biology 2:157–169.

Metcalfe, A. N. 2018. Aquatic insect distribution in the Colorado River Basin. MS Thesis, Northern Arizona University, Flagstaff, Arizona. (Available from: https://search.proquest.com/docview/2051910485)

Metcalfe, A. N. 2020. Locality based caddisfly (*Hydropsyche oslari*) sampling data and CO1 sequences from the southwestern United States, 2013–2016. United States Geological Survey, Reston, Virginia. (Available from: https://doi.org/10.5066/P93GMB1Y)

Miller, M. P., D. W. Blinn, and P. Keim. 2002. Correlations between observed dispersal capabilities and patterns of genetic differentiation in populations of four aquatic insect species from the Arizona White Mountains, USA. Freshwater Biology 47:1660–1673.

Muehlbauer, J. D., S. F. Collins, M. W. Doyle, and K. Tockner. 2014. How wide is a stream? Spatial extent of the potential "stream signature" in terrestrial food webs using meta-analysis. Ecology 95:44–55.

Múrria, C., N. Bonada, C. Ribera, and N. Prat. 2010. Homage to the virgin of ecology, or why an aquatic insect unadapted to desiccation may maintain populations in very small, temporary Mediterranean streams. Hydrobiologia 653:179–190.

Myers, M. J., F. A. H. Sperling, and V. H. Resh. 2001. Dispersal of two species of Trichoptera from desert springs: Conservation implications for isolated vs connected populations. Journal of Insect Conservation 5:207–215.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, New York.

Nishimura, N. 1967. Ecological study on net-spinning caddisfly, *Stenopsyche griseipennis* McLachlan II. Upstream-migration and determination of flight distance. Mushi 40:39–46.

Nishimura, N. 1981. Ecological studies on the net-spinning caddisfly, *Stenopsyche japonica*. Martynov. 5. On the upstream migration of adult. Kontyû 49:192–204.

Ross, H. H. 1944. The caddis flies, or Trichoptera, of Illinois. Illinois Natural History Survey Bulletin 23:1.

Olsen, J. B., B. G. Flannery, T. D. Beacham, J. F. Bromaghin, P. A. Crane, C. F. Lean, K. M. Dunmall, and J. K. Wenburg. 2008. The influence of hydrographic structure and seasonal run timing on genetic diversity and isolation-by-distance in chum salmon (*Oncorhynchus keta*). Canadian Journal of Fisheries and Aquatic Sciences 65:2026–2042.

Oswood, M. W. 1976. Comparative life histories of the Hydropsychidae (Trichoptera) in a Montana lake outlet. American Midland Naturalist 96:493–497.

Papadopoulou, A., I. Anastasiou, and A. P. Vogler. 2010. Revisiting the insect mitochondrial molecular clock: The mid-Aegean trench calibration. Molecular Biology and Evolution 27:1659–1672.

Pauls, S. U., H. T. Lumbsch, and P. Haase. 2006. Phylogeography of the montane caddisfly *Drusus discolor*: evidence for multiple refugia and periglacial survival. Molecular Ecology 15(8): 2153–2169.

Pederson, J., K. Karlstrom, W. Sharp, and W. McIntosh. 2002. Differential incision of the Grand Canyon related to Quaternary faulting—Constraints from U-series and Ar/Ar dating. Geology 30:739–742.

Petersen, I., Z. Masters, A. G. Hildrew, and S. J. Ormerod. 2004. Dispersal of adult aquatic insects in catchments of differing land use. Journal of Applied Ecology 41:934–950.

Poole, G. C. 2002. Fluvial landscape ecology: Addressing uniqueness within the river discontinuum. Freshwater Biology 47: 641–660.

Rader, R. B., P. J. Unmack, W. F. Christensen, and X. Jiang. 2019. Connectivity of two species with contrasting dispersal abilities: A test of the isolated tributary hypothesis. Freshwater Science 38:142–155.

Rodriguez-Iturbe, I., R. Muneepeerakul, E. Bertuzzo, S. A. Levin, and A. Rinaldo. 2009. River networks as ecological corridors: A complex systems perspective for integrating hydrologic, geomorphologic, and ecologic dynamics. Water Resources Research 45.

Schefter, P. W., and G. B. Wiggins. 1986. A systematic study of the nearctic larvae of the Hydropsyche morosa group (Trichoptera: Hydropsychidae). Life Sciences Miscellaneous Publications, Royal Ontario Museum, Toronto, Canada.

Schultheis, A. S., and J. M. Hughes. 2005. Spatial patterns of genetic structure among populations of a stone-cased caddis (Trichoptera: Tasimiidae) in south-east Queensland, Australia. Freshwater Biology 50:2002–2010.

Stevens, L. E., J. P. Shannon, and D. W. Blinn. 1997. Colorado River benthic ecology in Grand Canyon, Arizona, USA: Dam, tributary and geomorphological influences. Regulated Rivers: Research and Management 13:129–149.

Sweeney, B. W., J. M. Battle, J. K. Jackson, and T. Dapkey. 2011. Can DNA barcodes of stream macroinvertebrates improve descriptions of community structure and water quality? Journal of the North American Benthological Society 30:195–216.

Suzuki, T., T. Kitano, and K. Tojo. 2014. Contrasting genetic structure of closely related giant water bugs: Phylogeography of *Appasus japonicus* and *Appasus major* (Insecta: Heteroptera, Belostomatidae). Molecular Phylogenetics and Evolution 72:7–16.

Svensson, B. W. 1974. Population movements of adult Trichoptera at a South Swedish stream. Oikos 25:157–175.

Tonkin, J. D., J. Heino, and F. Altermatt. 2018. Metacommunities in river networks: The importance of network structure and connectivity on patterns and processes. Freshwater Biology 63:1–5.

Wallace, J. B., and R. W. Merritt. 1980. Filter-feeding ecology of aquatic insects. Annual review of Entomology 25:103–132.

Wickham, H., W. Chang, L. Henry, T. L. Pedersen, K. Takahashi, C. Wilke, K. Woo, H. Yutani, D. Dunnington, and R Development Team. 2020. *ggplot2*: Create elegant data visualisations using the grammar of graphics. (Available from: https://CRAN.R-project.org/package=ggplot2)

Wilke, T., R. Schultheis, and C. Albrecht. 2009. As time goes by: A simple fool's guide to molecular clock approaches in invertebrates. American Malacological Bulletin 27:25–45.

Woodbury, A. M., S. Flowers, D. W. Lindsay, S. D. Durrant, N. W. Dean, A. W. Grundmann, J. R. Crook, W. H. Behle, H. G. Higgins, G. R. Smith, G. G. Musser, and D. B. McDonald. 1959. Ecological studies of flora and fauna in Glen Canyon. Anthropological Papers, Glen Canyon Series no. 7. University of Utah Press, Salt Lake City, Utah.

Yaegashi, S., K. Watanabe, M. T. Monaghan, and T. Omura. 2014. Inferring fine-scale dispersal in the caddisfly *Stenopsyche marmorata* (Trichoptera; Stenopsychidae) using spatial autocorrelation of microsatellite markers. Freshwater Science 33: 172–180.