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RESEARCH ARTICLE

Multiple lines of genetic inquiry reveal effects of local and landscape factors on an amphibian metapopulation

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Abstract

Context A central tenet of landscape ecology is that both characteristics of patches and the matrix between them influence functional connectivity. Landscape genetics seeks to evaluate functional connectivity by determining the role of spatial processes in the distribution of genetic diversity on the landscape. However, landscape genetics studies often consider only the landscape matrix, ignoring patch-level characteristics, and possibly missing significant drivers of functional connectivity.

Objectives (1) Evaluate drivers of functional connectivity for an amphibian metapopulation, and (2) determine whether local characteristics are as important as landscape features to functional connectivity of this species.

Methods We used gravity models to evaluate the evidence for hypothesized drivers of functional connectivity for *Dryophytes wrightorum* that included both local and landscape attributes and a novel combination of methods of genetic inquiry: landscape genetics and environmental DNA (eDNA).

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Hypothesized drivers of connectivity included effects of hydrology, canopy cover, and species interactions.
Results Evidence weights indicated that stream networks were the most likely driver of functional connectivity, and connectivity along stream networks was positively correlated with gene flow. We also found a strong correlation between abundance of *D. wrightorum* from eDNA data and effective population size estimates from microsatellite data.
Conclusions We found evidence that functional connectivity of *D. wrightorum* was strongly driven by stream networks, despite considering multiple local and landscape processes. This suggests that management of this species focused on landscape hydrologic connectivity as gene flow corridors while maintaining current local management action is likely to have a positive effect on species conservation.

Keywords Arizona treefrog · *Dryophytes wrightorum* · Environmental DNA · Functional connectivity · Gravity model

Introduction

Functional connectivity, the realized degree of genetic flow through the landscape, is influenced by the spatial arrangement of an organism's habitat, its resource preferences or associations, and its ability to move through the landscape (With 1997; With et al. 1997). Understanding functional connectivity across a landscape is important for recognizing population dynamics such as genetic connectivity and gene flow (Hanski and Gilpin 1991). Additionally, functional connectivity analyses can be useful to inform conservation initiatives, especially for species in fragmented landscapes (Marsh and Trenham 2000). The field of landscape genetics combines population genetics and landscape ecology to understand how species' interactions with the landscape shape characteristics of their populations, with an emphasis on gene flow and resulting functional connectivity (Manel et al. 2003; Storfer et al. 2007).

Landscape genetic studies employ spatially explicit models to test specific hypotheses of how landscapes influence population processes (Balkenhol et al. 2009; Wagner and Fortin 2013). Functional connectivity assessments, particularly in landscape genetics, evaluate whether landscape attributes of the intervening matrix between sampling locations facilitate or inhibit functional connectivity such as gene flow (Ray 2005; McRae 2006; McRae et al. 2008). Patch-level characteristics have been shown to influence functional connectivity in combination with matrix attributes in a variety of taxa (Banks et al. 2005; Murphy et al. 2010; Dileo et al. 2014; Watts et al. 2015; Zero et al. 2017; Robertson et al. 2018), yet patch variables, or local characteristics, are considered less frequently in assessments of functional connectivity than landscape characteristics (Sork and Waits 2010; Pflüger and Balkenhol 2014).

Local habitat characteristics or patch-level species interactions such as predation, competition, or disease can influence functional connectivity through differential production of migrants or varying attractiveness to dispersing individuals (Fotheringham and O'Kelly 1989; Banks et al. 2005). What remains unclear is the relative contribution of local and landscape characteristics to functional connectivity for many species. Does the inclusion of local variables change our understanding of functional connectivity compared to landscape hypotheses alone? As a combination of local and landscape features likely influence functional connectivity, incorporating both local and landscape processes could better inform the most effective scale of management actions.

Amphibians are a key study organism for questions regarding functional connectivity (Storfer et al. 2010) and have been broadly studied in landscape genetics (Spear et al. 2005; Goldberg and Waits 2010; Storfer et al. 2010). Amphibian population processes occur at a scale tractable for landscape genetic study, they associate with discrete breeding habitats (patches), and there is a growing need to improve the conservation of this taxon given the global threats they face (Stuart et al. 2004; Storfer et al. 2009). However, like many other taxa, studies of amphibian functional connectivity have typically focused on landscape processes without accounting for local variables.

At the local scale, inter-and intraspecific interactions, such as predation or competition, may influence functional connectivity of a focal species through limiting population size or dispersal. However, community information can be difficult to obtain and therefore include in landscape genetics studies, especially for species that are cryptic or exhibit less predictable phenology like many amphibians

(Mazerolle et al. 2007). Other landscape genetic studies have used indirect estimates of species interactions, such as an environmental variable that covaries with the occurrence of species of interest (Murphy et al. 2010) or an index of predator densities from harvest data (Howell et al. 2016). Environmental DNA (eDNA) is an emerging technique that can be used to create multi-species datasets at the local level for landscape genetics studies. Environmental DNA is the detection of species via trace amounts of their DNA expelled into the environment (Rees et al. 2014). Although methods of eDNA analysis continue to be refined, it is already a powerful tool in ecological studies, especially for species that are not easily detected by traditional means (Ficetola et al. 2008; Goldberg et al. 2011; Biggs et al. 2015). Environmental DNA may provide information on local abundance of focal or interacting species, which can be used as part of analyses of functional connectivity. Assessing local abundance using eDNA data is at the forefront of development of eDNA methods and technology (Cristescu and Hebert 2018). The incorporation of eDNA data in a landscape genetics study can provide new insights, such as the dynamics of populations and interactions with predators, competitors, or invasive species on functional connectivity.

The Arizona treefrog, *Dryophytes (Hyla) wrightorum,* is a pond-breeding amphibian endemic to the arid southwestern United States and northern Mexico. Previous studies have demonstrated that the Huachuca Mountains Canelo Hills (HMCH) population segment of *D. wrightorum* is genetically isolated from the two larger populations, exhibiting differences in morphology and advertisement calls when compared to individuals in the rest of their range (Gergus et al. 2004a). Populations in this region have significant degrees of genetic subdivision and behave as a metapopulation (Mims et al. 2016). Additionally, the HMCH region has relatively few populations of *D. wrightorum* (N < 20) and few predators exist in the area (but see Jones and Timmons 2010). The genetic and morphological distinctions of this population segment, along with its small and geographically restricted nature, led to its review as a candidate for listing under the U.S. Endangered Species Act as a Distinct Population Segment, though it has since been designated as not meriting federal protection (USFWS 50 CFR Part 17 2016). A recent genetic study evaluated four hypothesized drivers of functional connectivity assessing landscape characteristics (isolation by distance, isolation by slope, connectivity by canopy, and connectivity by stream) and found the most support for isolation by distance in their analysis, though there was some evidence for isolation by slope and connectivity by canopy (Mims et al. 2016). However, this work did not consider local characteristics.

To investigate the relative contribution of local and landscape characteristics on functional connectivity, we evaluated a comprehensive suite of hypothesized relationships between *D. wrightorum* genetic structure and variables at the local and landscape scale. Of particular interest was whether the inclusion of local habitat and species interaction variables would alter our understanding of this species and its functional connectivity relative to the previous analysis of landscape attributes alone. We hypothesize that functional connectivity of this species may be influenced by a large suite of ecologically and biologically relevant processes influencing *D. wrightorum* in this area. These hypotheses include the influence of hydrology at ponds and throughout the landscape, topographical barriers, vegetation, pond network dynamics, and presence of predators, pathogens, and competitors, as well as local abundance of *D. wrightorum* itself (Table 1). This research will help inform the relative role of local and landscape characteristics in functional connectivity and have applied value for conservation and management for *D. wrightorum*. We combined eDNA-derived sympatric, interacting species abundances, local and landscape variables, and population genetic data into a gravity modelling framework (Murphy et al. 2010) to answer the following questions: (1) What hypothesized model of functional connectivity is best supported for this amphibian? (2) Will these findings differ from those of Mims et al. (2016), which considered only landscape factors and their influence on functional connectivity?

Methods

Study area

The range of *D. wrightorum* is separated into three distinct portions: the Mogollon Rim of central Arizona and western New Mexico, the Sierra Madre Occidental of eastern Sonora and western Chihuahua, and the

Table 1 Hypotheses names, parameters, predicted direction of relationship between variables and gene flow, and ecological justification tested using gravity models for functional connectivity of *D. wrightorum*

Hypothesis	Parameters	Justification
Predation	Edges: None Nodes: Bullfrog (−)	Predators at ponds may reduce larval and adult survival at a site and subsequent gene flow (Rosen and Schwalbe 1995)
Water availability	Edges: Mean cti (+) Nodes: Hydroperiod (+), pond area (+)	Amphibians are physiologically water limited at breeding sites and across the landscape, especially in desert environments (Thorson 1955; Chew 1961)
Hydroperiod	Edges: None Nodes: Hydroperiod (+)	Given the unpredictable nature of precipitation events in the HMCH region (Higgins et al. 1997), the availability of water at breeding sites is likely an important factor in breeding, larval development, and the stability of local subpopulations, as has been shown for other arid-land amphibians (Pechmann et al. 1989; Amburgey et al. 2012)
Landscape moisture	Edges: Mean cti (+) Nodes: None	Amphibians have stringent water requirements (Buckley and Jetz 2007) and must conserve water when dispersing in a terrestrial environment due to desiccation risk (Thorson 1955)
Stream distance	Edges: Streamdist (−) Nodes: None	Dispersal may occur along stream corridors to avoid desiccation (Thorson 1955; Burbrink et al. 1998; Trumbo et al. 2013)
Topography	Edges: Max slope (−) Nodes: rsp (+)	Topography may act as a barrier to gene flow in amphibian species (Funk et al. 2005) such as steep slopes (Richards-Zawacki 2009). Ponds higher in rsp may produce more migrants that settle in lower ponds by means of easier movement downhill than uphill (Oldham 1967; Malonza and Measey 2005; Measey et al. 2007) or by flash flooding caused by monsoons
Stepping stone	Edges: None Nodes: Betweenness (+)	Wetlands within a network may act as stepping stones, facilitating dispersal among occupied wetlands (Fortuna et al. 2006)
Connectedness	Edges: None Nodes: Degree (+)	Wetland structural connectivity is important in determining the pattern of functional connectivity in some amphibians (Ribeiro et al. 2011). Therefore, a particular breeding pond's level of connectedness within a larger network of all intermittent wetlands in the HMCH region may influence functional connectivity of *D. wrightorum*
Vegetation productivity	Edges: SAVI (+) Nodes: SAVI (+)	Vegetation along network edges should facilitate dispersal by inhibiting solar radiation and evaporation, lowering the threat of desiccation and increasing gene flow (Spear and Storfer 2008; Mims et al. 2016). At the node level, *D. wrightorum* is associated with abundant vegetation at breeding sites (Stebbins 1962; Gergus et al. 2004b)
Productivity	Edges: None Nodes: hli (+), SAVI (+)	Primary productivity can be a constraint in species richness (Buckley and Jetz 2007) and dispersal (Field et al. 2009). *D. wrightorum* is associated with breeding sites with abundant vegetation (Stebbins 1962; Gergus et al. 2004b) and hli has been used by others to estimate patch-level productivity (Murphy et al. 2010)
Isolation by distance (IBD)	Edges: None Nodes: None	The null expectation should conform to isolation by distance given the limited dispersal ability, strong site fidelity of many amphibians (Berven and Grudzien 1990; Pittman et al 2008), and support for IBD from Mims et al. (2016)
Abundance null	Edges: None Nodes: DYWR eDNA (+)	Larger populations may produce more migrants and act as source populations (Pulliam 1988)
Global	Edges: Max slope (−), mean cti (+), mean SAVI (+), streamdist (−) Nodes: Bullfrogs (−), hli (+), DYWR eDNA (+), pond area (+), rsp (+), betweenness (+)	Functional connectivity of *D. wrightorum* is influenced by a combination of all variables tested

In addition to edge and node variables, Euclidean distance is also included in each model. Predicted relationships denoted as (+) for increasing gene flow or (−) for impeding gene flow. DYWR eDNA denotes *D. wrightorum* eDNA abundance estimates

intervening small, isolated HMCH region in southeastern Arizona (Duellman 1970). The HMCH region is neighbored by several mountain ranges of mostly arid scrubland with variable elevation, precipitation patterns, vegetation, and temperature (Gehlbach 1993). In this area, *D. wrightorum* is associated with streams, wetlands, and man-made cattle stock ponds (Mims et al. 2016). Currently, management of ponds

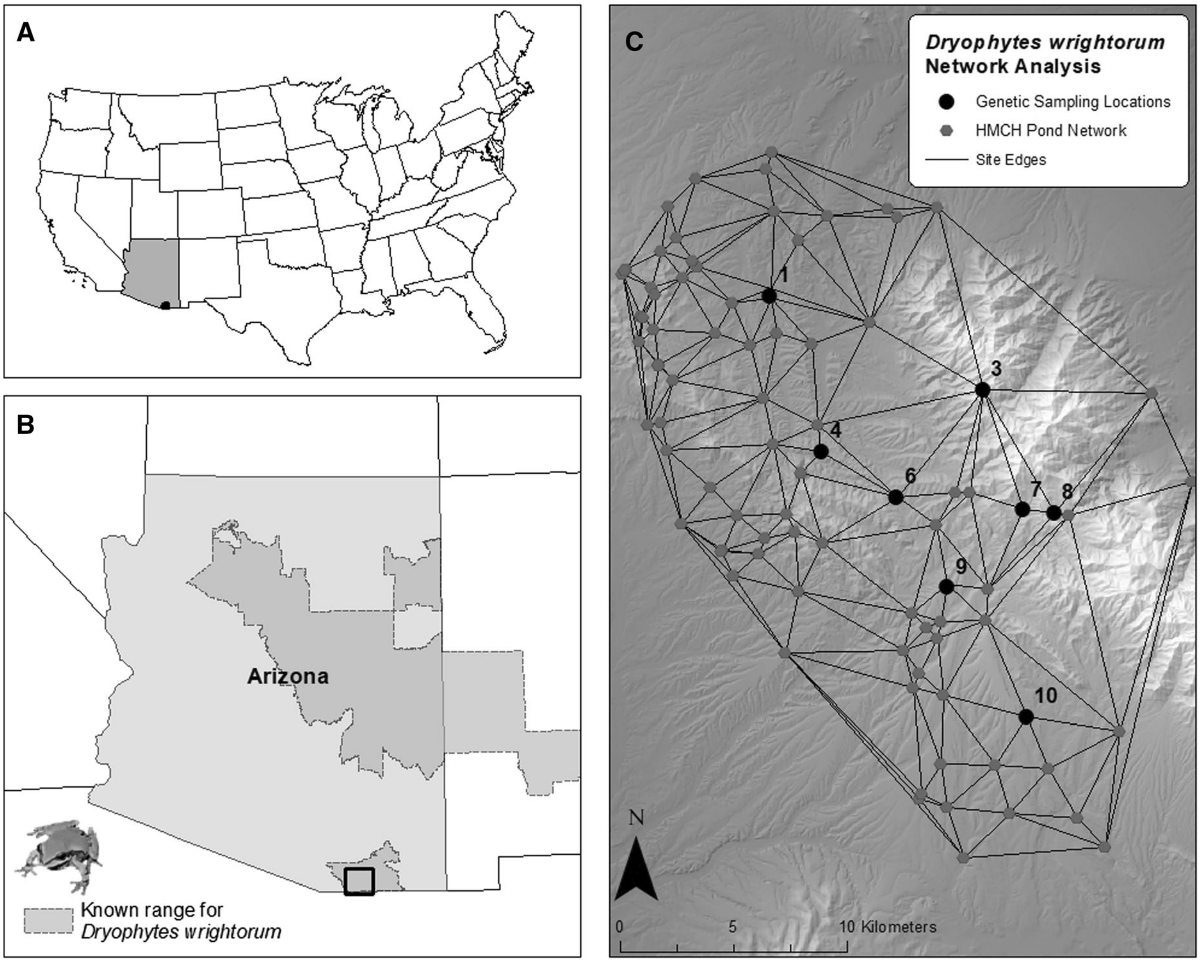

Fig. 1 Known geographic extent for *D. wrightorum* Arizona, U.S.A. (IUCN, A, B). The 91 ponds found within the HMCH region (C) were used in the network analysis and include the eight sites from which genetic and eDNA sampling occurred (population numbers matched to the 10 sites assessed by Mims et al. (2016))

in this area including hydroperiod alteration and removal of invasive species such as the American bullfrog is being conducted in order to support native herpetofauna (John Kraft, U.S. Forest Service, personal comm.).

Genetic data

We analyzed population genetic and eDNA data collected in 2014 at eight sites in the HMCH region (Fig. 1) of southern Arizona by Mims et al. (2016) and Goldberg et al. (2018), respectively. We used previously generated data for 17 microsatellite loci of 215 individuals with a mean sample size of 27.6 per site (sample size range: 19–40 individuals per site, see Mims et al. 2016). Loci were screened for linkage disequilibrium and deviations from Hardy–Weinberg equilibrium with Bonferroni corrections using GenePop 1.2 (Raymond and Rousset 1995) and for full siblings using COLONY 2.0 (Jones and Wang 2009; Goldberg and Waits 2010). For any full sibling pairs detected, one member was randomly chosen to be removed (Goldberg and Waits 2010). We calculated pairwise proportion of shared alleles (D_{PS}, Bowcock et al. 1994), fixation indices (F_{ST}, Weir and Cockerham 1984), and Slatkin's linearized F_{ST} (linF_{ST}, Slatkin 1995). Additionally, effective population size (N_e) for each sampling location was estimated using the linkage disequilibrium method (LDNe, Waples and Do 2008) in NeEstimator V2 (Do et al. 2014; Mims et al. 2016).

Environmental DNA sampling was performed concurrently with genetic sampling of *D. wrightorum*. Four replicate 250 mL water samples were filtered from one location in each wetland using 0.45 µm cellulose nitrate single-use sterile filter funnels (Whatman^{TM}, GE Healthcare, Pittsburgh, PA; Goldberg et al. 2018). DNA was then extracted from the filters following best practice protocols (Goldberg et al. 2016) using the Qiashredder and DNeasy method described in Goldberg et al. (2011). Samples were analyzed using species-specific qPCR, and negative controls were included at filtering, extraction, and PCR stages to test for contamination. Additionally, a positive control (IPC; Applied Biosystems, Foster City, CA) to test for inhibition was included in each reaction. Species-specific qPCR assays were used to survey sites for *D. wrightorum* (Goldberg et al. 2018), invasive American bullfrogs (*Lithobates catesbeianus*; Strickler et al. 2015), tiger salamanders (*Ambystoma mavortium*; Goldberg et al. 2018), virile crayfish (*Orconectes virilis*; Table S1), and the fungal pathogen Bd (*Batrachochytrium dendrobatidis*; Boyle et al. 2004). We used the concentration of eDNA estimated for each sample (averaged across the four replicates) as an index of species abundance at each site via quantitative standards for each species. Quantitative standard curves were created using diluted DNA samples from tissue in duplicate diluted from 10^{-3} to 10^{-6}, except in the case of Bd where standards were created using gBlocks (Integrated DNA Technologies, Coralville, IA) from 10^4 to 10^1.

Evaluation of population size estimates from genetic sampling

In this study system, traditional population genetic surveys and quantitative eDNA analysis were conducted from simultaneously-collected samples in the same localities, a rare occurrence that provides the opportunity for additional analyses. We hypothesized that eDNA concentrations correlate with local abundance of *D. wrightorum* at a site, though we did not have census population size data to assess this relationship. To determine the validity of using eDNA concentrations as a surrogate for local abundance, we instead evaluated the relationship between eDNA concentrations and N_e estimates from microsatellite data. We tested for a relationship between these two data types (eDNA concentrations of *D. wrightorum* log transformed for normality) via Pearson product-moment correlation (Pearson 1896) using R version 3.6.1 (R Core Team 2019).

Landscape and spatial data

We collected landscape and spatial information related to our hypotheses that included landscape data, local variables, and pond-network variables that spanned ecologically relevant processes associated with *D. wrightorum* biology and the landscape of the HMCH region (Table 1; Table S2). We utilized a 30 m resolution digital elevation model (DEM) from the National Elevation Dataset (U.S. Geological Survey 2017) to derive various topographic measures. From the DEM, we calculated heat load index (hli, McCune and Keon 2002; 'spatialEco' 0.0.1–7 Evans 2017; R 3.5.0 R Core Team 2018), compound topographic index (cti, Moore et al. 1993; Gessler et al. 1995; ArcMap 10.4.1 ESRI, Geomorphometry and Gradient Metrics Toolbox 2.0 Evans et al. 2014), slope (ArcMap 10.4.1 ESRI, Geomorphometry and Gradient Metrics Toolbox 2.0 Evans et al. 2014), and relative slope position (rsp, Berry 2002; ArcMap 10.4.1 ESRI, Geomorphometry and Gradient Metrics Toolbox 2.0 Evans et al. 2014). Additionally, we gathered Tier 1 Landsat satellite scenes over 4-years (Landsat 5: September 16 2010, September 19 2011, Landsat 8: September 24 2013, September 30 2015), targeting dates for each year near the end of the monsoon season when vegetation is most extensive and when cloud cover was not present. We standardized the LT05 scenes to the LC08 scenes using the Apply Gain and Offset function in the remote sensing software ENVI version 5.3.1 (Exelis Visual Information Solutions, Boulder, Colorado). We calculated Soil-Adjusted Vegetation Index (SAVI; Huete 1988) from the Landsat scenes (soil reflectance set to 0.50) to assess plant productivity while accounting for soil reflectance and averaged SAVI values across the 2010–2015 time period for modelling.

We extracted data from raster layers for landscape variables by taking the mean (SAVI and cti) or maximum (slope) value along a buffered straight-line connecting each pair of populations. To examine the effect of buffer size, we extracted edge data at three buffer widths (30 m, 120 m, and 300 m) using 'GeNetIt' (v 0.1–2 Murphy et al. 2010, R 3.6.1 R Core Team 2019) and evaluated pairwise correlations between the extracted data for each buffer width. We found edge values to be highly correlated ($r > 0.74$) across buffer widths, and we thus applied a single buffer width of 30 m for between-site variables in building models as that scale is most relevant to *D. wrightorum* ecology (as in Robertson et al. 2018). Next, we extracted data from raster layers for local measures of SAVI and hli by calculating the mean of the data from a 100 m radius around the coordinates of the centroid of each wetland and rsp at the centroid point of each wetland with no buffer using 'GeNetIt' (v 0.1–2 Murphy et al. 2010, R 3.6.1 R Core Team 2019).

We used the National Hydrography Dataset (NHD, US Geological Survey 2013) to produce a pairwise stream distance matrix (stream network distance) between each of our sites. Stream network distance represents the shortest two-dimensional distance between each sampling location using stream network connections as paths. We used QGIS version 2.18 (QGIS Development Team 2018) to trim the NHD stream network across the study area to include only reaches that were relevant to the connections among sites and then created a pairwise distance matrix between all sites using the package 'riverdist' (v 0.15.0 Tyers 2017, R 3.5.0 R Core Team 2018). Two sampling locations (Ponds 7 and 9) are located in watersheds that are part of a different river basin (the San Pedro River Basin) than the other sites (the Santa Cruz River Basin). For that reason, we assigned the stream network distance as one order of magnitude larger than the furthest stream distance of sites in the same river basin to avoid extraneous pairwise distance comparisons with those two sites (as these two river basins do not converge for > 1000 km).

To capture more hydrological detail on the small, intermittent ponds used by *D. wrightorum* for breeding, we created a wetland network of ponds in the HMCH region. We coupled a US Forest Service Ranger District Map, which reliably reports stock pond locations, with Google Earth imagery (2014) to manually create a digital pond database for the Huachuca Mountains and its drainages (Fig. 1). We used Google Earth to create polygons representing pond basins. We then used historical satellite imagery available via the Google Earth Historical Imagery tab (Map data: DigitalGlobe, USGS, USDA Farm Service Agency) to construct a time series of when ponds contained water (Figure S1). The wet/dry historical records for ponds were then used to determine whether ponds and lakes were intermittent (dry in at least one historical image) and potential breeding sites for *D. wrightorum*. All perennial ponds (those always observed with water) and dry basins (those never observed to hold water) were excluded from the candidate set of breeding ponds for the wetland network. All intermittent ponds were considered candidate ponds for use by *D. wrightorum* and included in the network. We defined the study's spatial focal area as all intermittent ponds within a 7 km buffer of all known breeding sites for *D. wrightorum* in the Huachuca Mountains, as this is the longest distance between known breeding sites of *D. wrightorum* in this range.

To parameterize network location of sampled sites, we conducted a graph network analysis using the wetland network dataset that totaled 91 wetlands, including our eight sampling locations. We created a Delaunay triangulation network from our wetland network (Fig. 1; 'deldir' v 0.1–15 Turner 2018, R 3.6.1 R Core Team 2019). We calculated two node-based graph metrics: degree (the number of connections a node has) and betweenness (the number of times a node is crossed in the shortest path between two other nodes) in 'igraph' (v 1.2.1 Csardi and Nepusz 2006, R 3.6.1 R Core Team 2019) and extracted both of these values for our eight sampling locations of interest.

For each of our eight sites with population genetic data, we estimated the stability of breeding ponds during breeding season using Google Earth (Map data: Google, DigitalGlobe, USGS). We calculated a hydroperiod index as the percent of images a pond was observed to have water during the months of July through November (the primary breeding and larval development season for *D. wrightorum*) across all years of imagery available in our study area (1992–2014; Figure S1). Additionally, we calculated the surface area of each pond from Google Earth imagery at maximum water height in the historic images available occurring in 2003, 2006, 2013, and 2015 depending on the pond location. We chose the imagery where ponds appeared largest to estimate pond size area around the occurrence of monsoon season; this is thus reflective of the maximum amount of habitat available when *D. wrightorum* are breeding and developing in those ponds.

Gravity models

We used gravity models (Anderson 1979; Fotheringham and O'Kelly 1989), a specific type of network model, to assess how characteristics of breeding ponds and the intervening landscape influence functional connectivity of *D. wrightorum* in the HMCH region. This approach models gene flow (1-genetic distance) as a function of Euclidean distance between sites and a combination of local characteristics that influence the production of migrants in the network and landscape characteristics that limit or facilitate migration between nodes (Murphy et al. 2010). Singly constrained gravity models include distance, local, and landscape parameters as fixed effects and sites as random effects to account for the non-independence between sites (Murphy et al. 2010; Robertson et al. 2018). For solving, the multiplicative, exponential gravity model form is natural log transformed for linearity, then estimated with a linear mixed effects model.

Prior to modeling, we scaled each of our variables by standard deviation and centered them at zero using R version 3.6.1 (R Core Team 2019). In order to avoid negative or zero values that result in missing data when the gravity model is log transformed, we added the absolute value of each scaled variable's minimum plus 0.001 to all data values of the variable; with this, the minimum value of each variable was 0.001. Additionally, we evaluated our scaled data for collinearity and multicollinearity using Pearson product correlations and variable inflation factor (VIF; Mansfield and Helms 1982). We did not include collinear variables ($r > 0.70$) or multicollinear variables ($\mathrm{VIF} > 5$) in the same model (Table S3).

After removing correlated variables from each model, we performed singly-constrained gravity models using 'GeNetIt' (v 0.1–2 Murphy et al. 2010, R 3.6.1 R Core Team 2019). We specified sites as producers (producing migrants) on a saturated network, which included connections to and from every site. We evaluated model rankings for each estimate of gene flow (1-D_{PS}, 1-F_{ST}, and 1-linF_{ST}) using maximum likelihood (ML) estimates of Akaike information criterion scores adjusted for small sample size (AIC_C), Bayesian information criterion (BIC) values, and evidence weights of each for model ranking (Akaike 1973; Burnham and Anderson 2002). Finally, we used gravity models evaluated with restricted maximum likelihood (REML) to determine parameter estimates of our top models for each gene flow metric (Zuur et al. 2009; Zero et al. 2017). Gravity models calculate parameter estimates and confidence intervals from log transformed data and are presented as transformed values here.

Results

Genetic data

A total of 215 individuals were genotyped using microsatellites, and 32 eDNA filters were analyzed for

Table 2 Environmental DNA sampling results from the eight sampling locations

Sampling location	DYWR (ng/L)	Bullfrog (pg/L)	Bd (copy number)	Tiger salamander (ng/L)	Virile crayfish (ng/L)
1	0.248	3.454	0.000	0.000	0.000
3	18.703	0.000	0.000	0.000	0.000
4	0.050	1.841	2133.3	0.000	0.000
6	0.666	0.749	0.000	0.000	0.000
7	0.956	0.000	0.000	0.000	0.000
8	0.518	0.000	0.000	0.000	0.000
9	3.811	0.000	0.000	0.000	0.000
10	0.031	0.000	0.000	0.000	0.000

Results are listed as eDNA concentrations normalized by quantitative standards produced independently for each species (DYWR denotes *D. wrightorum*), or as copy number for *Batrachochytrium dendrobatidis* (Bd)

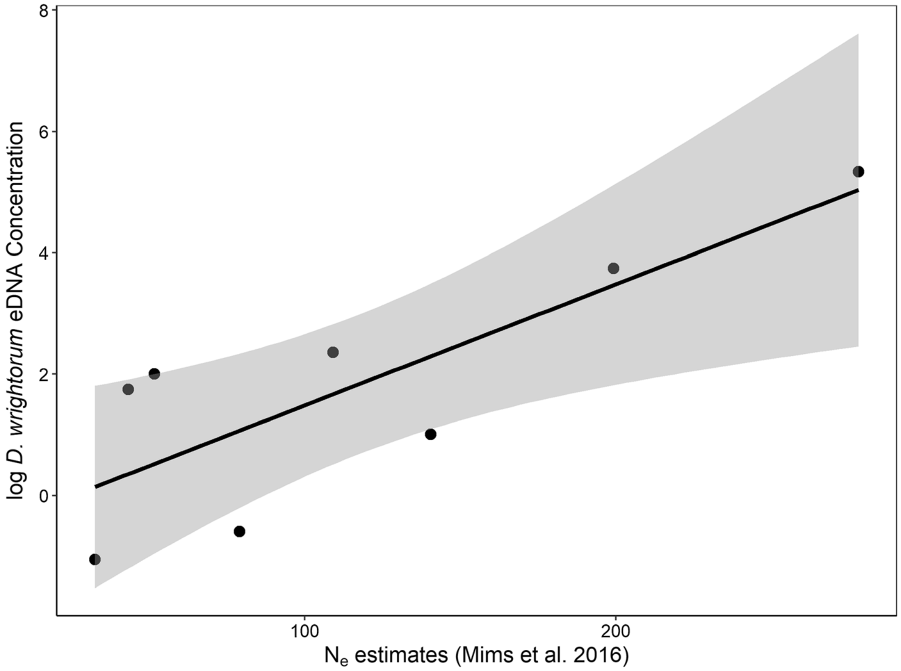

Fig. 2 Correlation (r = 0.812, p = 0.014) between qPCR-estimated *D. wrightorum* eDNA concentrations based on a quantitative standard curve, created from diluted tissue samples and N_e estimates from microsatellite data (Mims et al. 2016), with best fit line and 95% confidence interval shaded in gray

the presence of five different species. Microsatellite loci screening for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium showed inconsistencies across populations and insufficient evidence for removal of loci from analysis (Mims et al. 2016). There were only five cases of significant deviations from Hardy–Weinberg equilibrium after Bonferroni correction occurring across five different loci and three different populations. Similarly, linkage disequilibrium was observed in two marker pairs in only one population. Effective population sizes of the eight sampling sites ranged from 32.5 to 277.9 individuals with an average of 116.65 individuals. Pairwise genetic distances between the eight populations ranged from 0.212–0.392 for D_{PS}, 0.0086–0.0891 for F_{ST}, and 0.009–0.098 for linF_{ST}. For complete population genetic analyses of sampling locations using these microsatellite markers, see Mims et al. (2016).

There were few or no eDNA-detected presences in three out of five sampled species (Bd, tiger salamanders, virile crayfish). We did not detect tiger salamanders or virile crayfish in any of the eight sampling locations, and we detected Bd in low concentration at only one site (Table 2). As a result, we only included *D. wrightorum* and bullfrog eDNA data in our analyses. *Dryophytes wrightorum* were detected at all eight sampling sites at concentrations ranging from 0.031–18.703 ng/L. Bullfrogs were detected at three of eight sampling sites at concentrations ranging from no detection to 3.454 pg/L. Quantitative PCR efficiencies ranged between 95 and 105% and standard curves had $r^2 \geq 0.98$.

We found a strong positive correlation between eDNA concentrations of *D. wrightorum* and N_e estimates from each site (r = 0.81, $p = 0.01$; Fig. 2). Based on this result, we determined that eDNA abundance estimates for *D. wrightorum* were an appropriate surrogate for local abundance, a local variable used in the abundance null model.

Local and landscape data

The local and landscape data collected for use in gravity modelling showed high levels of variation among the eight sampling locations (Table S2). Pairwise Euclidean distances between ponds ranged from 1.381 to 21.958 km, while pairwise stream network distances ranged from 8.016 to 189.571 km within the

Santa Cruz River Basin (excluding Ponds 7 and 9). Maximum slope between sites ranged from 18.605–41.942°. Additionally, there was notable variation in pond area (365.33–55,962.95 m^2) and hydroperiod index (0.17–0.82).

We found significant correlations ($r > 0.70$) between three pairs of local variables. Two graph network metrics, degree and betweenness, were correlated ($r = 0.91$) along with hydroperiod index and the local SAVI measure ($r = 0.74$). Additionally, local SAVI was positively correlated with *D. wrightorum* abundance estimates from eDNA sampling ($r = 0.81$). There were no significant correlations among the landscape variables used in our gravity models. To avoid collinearity and multicollinearity, we omitted local SAVI, degree, and hydroperiod from the global model (Table S3). Correlated variables did not occur together in our hypothesized models, causing no further omissions.

Gravity models

Model rankings were similar between our three measures of gene flow (Table 3). The stream distance model was the most supported based on AIC_C and BIC rankings for all three measures of gene flow and accounted for around half of the evidence weight in each of our model sets (Table 3). Within this model, both Euclidean distance and stream network distance significantly impeded gene flow, with 95% confidence intervals that did not overlap zero (Table 4).

The predation model was ranked higher than the isolation by distance model according to AIC_C but lower according to BIC for all measures of gene flow (evidence weights 0.13 and 0.12 respectively, Table 3). Within this model, Euclidean distance significantly impeded gene flow, similar to the stream distance model (Table 4). Bullfrog presence facilitated gene flow, though not significantly in this model (95% confidence interval overlapping zero, Table 4). Second to stream distance, the isolation by distance model was more highly supported than all other candidate models in our model set according to BIC ranking (0.18–0.20 evidence weight, Table 3).

There was little support for hypothesized relationships between gene flow and topographic variables, parameters relating to productivity, other measures of water availability, or graph network statistics

Table 3 Functional connectivity models, total number of parameters in each model (K), and ML estimated results ranked by ΔAIC_C of D_{PS}

Model	K	$1\text{-}D_{PS}$				$1\text{-}F_{ST}$				1-linF_{ST}			
		ΔAIC_C	AIC_C Weight	ΔBIC	BIC Weight	ΔAIC_C	AIC_C Weight	ΔBIC	BIC Weight	ΔAIC_C	AIC_C Weight	ΔBIC	BIC Weight
Streamdist	2	0.00	0.43	0.00	0.42	0.00	0.51	0.00	0.49	0.00	0.51	0.00	0.49
Predation	2	2.45	0.13	2.45	0.12	2.75	0.13	2.75	0.12	2.67	0.13	2.67	0.13
IBD	1	3.47	0.08	1.45	0.20	4.02	0.07	2.00	0.18	3.98	0.07	1.96	0.18
Hydroperiod	2	3.39	0.08	3.39	0.08	5.41	0.03	5.41	0.03	5.37	0.03	5.37	0.03
Stepping stone	2	3.81	0.06	3.81	0.06	5.36	0.04	5.36	0.03	5.30	0.04	5.30	0.03
Landscape moisture	2	4.59	0.04	4.59	0.04	4.33	0.06	4.33	0.06	4.35	0.06	4.35	0.06
Vegetation	3	5.18	0.03	7.20	0.01	5.55	0.03	7.58	0.01	5.57	0.03	7.60	0.01
Global	11	5.25	0.03	23.48	0.00	5.46	0.03	23.69	0.00	5.37	0.03	23.60	0.00
Connectedness	2	5.26	0.03	5.26	0.03	5.63	0.03	5.63	0.03	5.59	0.03	5.59	0.03
Abundance null	2	5.41	0.03	5.41	0.03	6.02	0.03	6.02	0.02	5.98	0.03	5.98	0.02
Water	4	5.60	0.03	9.65	0.00	7.03	0.02	11.08	0.00	7.01	0.02	11.06	0.00
Topography	3	6.90	0.01	8.93	0.00	7.61	0.01	9.64	0.00	7.54	0.01	9.56	0.00
Productivity	3	7.39	0.01	9.42	0.00	7.85	0.01	9.87	0.00	7.80	0.01	9.83	0.00

Euclidean distance is included as a parameter in each model and accounted for in K, though not listed individually for each

Table 4 Parameter estimates, standard errors (SE), and 95% confidence intervals (CI) for the top performing models based on AIC$_C$ and BIC ranking, evaluated using REML

Gene flow measure	Parameter	β	SE	95% CI	
Stream distance model					
1-D$_{PS}$	Intercept	− 0.3480	0.0111	− 0.3697	− 0.3263
	Stream Distance	− 0.0077	0.0033	− 0.0142	− 0.0012
	Distance	− 0.0147	0.0051	− 0.0247	− 0.0047
1-F$_{ST}$	Intercept	− 0.0439	0.0040	− 0.0518	− 0.0359
	Stream Distance	− 0.0028	0.0011	− 0.0051	− 0.0006
	Distance	− 0.0040	0.0018	− 0.0075	− 0.0006
1-linF$_{ST}$	Intercept	− 0.0465	0.0045	− 0.0553	− 0.0378
	Stream Distance	− 0.0031	0.0013	− 0.0057	− 0.0006
	Distance	− 0.0044	0.0020	− 0.0083	− 0.0005
Predation model					
1-D$_{PS}$	Intercept	− 0.3199	0.0154	− 0.3500	− 0.2897
	Bullfrogs	0.0046	0.0028	− 0.0009	0.0101
	Distance	− 0.0156	0.0053	− 0.0260	− 0.0052
1-F$_{ST}$	Intercept	− 0.0332	0.0056	− 0.0442	− 0.0222
	Bullfrogs	0.0018	0.0010	− 0.0002	0.0038
	Distance	− 0.0043	0.0018	− 0.0079	− 0.0007
1-linF$_{ST}$	Intercept	− 0.0347	0.0062	− 0.0468	− 0.0226
	Bullfrogs	0.0020	0.0011	− 0.0002	0.0042
	Distance	− 0.0047	0.0021	− 0.0088	− 0.0007

Parameter estimates and confidence intervals shown were calculated from log transformed, standardized data, and have not been back transformed

(Table 3). Additionally, our abundance null model containing distance and *D. wrightorum* eDNA estimates to represent the species' abundance at a site was not highly supported in this framework (Table 3).

Discussion

Our results highlight the utility of applying multiple analytic methods and including both local and landscape characteristics to inform the functional connectivity of species across a landscape. By analyzing a more complete set of hypotheses, relative importance of local and landscape attributes can be determined and better inform management action to the most pertinent scale. We found that stream network distance is the most supported driver of functional connectivity for *D. wrightorum* when competed against a suite of both local and landscape characteristics in our hypotheses. Based on our analysis for this species, landscape-level connectivity along stream corridors may be more important than local factors. However, it is worth noting that our limited sample size may not have captured all biologically important variables. We also found a novel correlation between eDNA abundance estimates of *D. wrightorum* and N$_e$ estimates from microsatellite genetic data. This finding provides new insight on the dynamics of populations and allowing assessment of local variables and other demographic processes not captured in modelling of functional connectivity alone.

Stream distance best explains functional connectivity of *D. wrightorum*

We found support for stream distance as the determinant driver of *D. wrightorum* functional connectivity within the HMCH region. Stream corridors provide access to water and vegetative cover to dispersing individuals that may aid in preventing desiccation and mortality when traversing the landscape (Thorson 1955; Pilliod et al. 2015). Considering the study area is in the arid southwestern United States, which exhibits variable precipitation patterns (Higgins et al. 1997), water is a limiting resource for *D. wrightorum* survival and dispersal. However, there was not support for models including other direct measures of water availability (water model, hydroperiod model, landscape moisture model) or vegetation (vegetation model). Additionally, the NHD used in creating this model is at a 1:100,000 scale which is likely coarser than what *D. wrightorum* are experiencing on the landscape. This suggests that either *D. wrightorum* individuals are using more prominent stream corridors for dispersal, or that river basins play an important role in shaping functional connectivity through other processes.

The finding that stream network distance was highly supported as an explanatory variable for functional connectivity, is contrary to results of resistance modelling on this same dataset (Mims et al. 2016). In the previous study, a resistance surface was created by buffering streams in the study area by 100 m and applying a low resistance value (1) within stream buffers and a high resistance value (100) outside of stream buffers, representing the hypothesis of low resistance to dispersal along riparian corridors for *D. wrightorum*. Using this method, models of isolation-by-distance and slope influencing functional connectivity received more support than that for stream corridors. The difference in results while using the same genetic dataset is likely due to major differences between the two methods. Even within a single method, differing parameterization of resistance surfaces can influence outcomes (Peterman et al. 2019). Therefore, major differences between gravity models and resistance surfaces such as model transformations, model composition, or parameterization of the landscape could explain differences in these results.

While the stream distance model better explained functional connectivity than the isolation by distance model, it is clear that Euclidean distance between ponds plays a large role in the functional connectivity of *D. wrightorum*. These results support the findings in Mims et al. (2016), which found that distance was a strong driver in *D. wrightorum* connectivity. Isolation by distance is common in ectothermic animals (Jenkins et al. 2010), especially amphibians (Beebee 2005), and distance has been shown to influence the genetic structure of other amphibian species in the same region (Mims et al. 2015).

eDNA and landscape genetics

Prior to the incorporation of eDNA into our modelling, we were interested in whether eDNA abundance estimates, based on concentration values, could be a proxy for local abundance of *D. wrightorum* and other sympatric, possibly interacting species. Environmental DNA is becoming a widely used tool for conservation and management applications (Goldberg et al. 2016), with the frontier of eDNA research aiming to gain population-level information from environmental samples. Many studies have attempted to relate eDNA abundance to density or biomass estimates (Pilliod et al. 2013; Biggs et al. 2015; Klymus et al. 2015); however, published relationships indicate low predictive power. To evaluate this, we assessed the relationship between eDNA and N_e estimates from microsatellite data and found a high level of correlation between the two. This shows an interesting convergence of different genetic techniques currently used to estimate local abundance and provides new insight toward the usefulness of eDNA as a tool for understanding populations.

To our knowledge, this is the first study to incorporate eDNA data as explanatory variables into landscape genetics research. While we did not find support for the influence of these species with regard to functional connectivity, *D. wrightorum* abundance estimated from eDNA was positively correlated with our measure for local vegetation (SAVI). Though these variables separately were not shown to significantly influence functional connectivity in the gravity model framework, there was some evidence that canopy cover influences functional connectivity by Mims et al. (2016); this relationship may be indicative of other local demographic processes. For example, this correlation may indicate how *D. wrightorum* uses habitat for dispersal, especially considering the species' reliance on emergent aquatic vegetation for calling and laying egg masses (Stebbins 1962). Local vegetation may generally be more indicative of *D. wrightorum* abundance at wetlands instead of explaining gene flow across the landscape, which provides valuable insight to the ecology of the species and can be used to inform conservation management.

There was some support for the predation model, indicating that bullfrog presence may influence functional connectivity in this species. However, the direction of bullfrog influence in the model was opposite of what we expected (positive effect on gene flow). This result is surprising, given the substantial negative effects of bullfrogs upon native herpetofauna in the region (Schwalbe and Rosen 1988; Rosen and Schwalbe 1995) and may indicate that this variable is collinear with another factor that was not measured or may reflect a process other than predation. For example, other anuran species have shown significant morphological and behavioral differences in the presence of predators (Lardner 2000; Relyea and Werner 2000; Relyea 2002). Though increased dispersal from ponds housing predators have not been directly shown, there is evidence for the possibility of this through morphological changes such as developing longer legs (Relyea 2001). Alternatively, the presence of bullfrogs may produce a sink population that could be forcing individuals to disperse. It is worth noting that only three of the eight sites in our analysis were positive for bullfrog eDNA, and we may have lacked the statistical power to reveal subtle effects of bullfrog abundance upon *D. wrightorum* functional connectivity.

Despite the promise of eDNA to evaluate community assemblages and include species interactions such as predation into landscape genetics studies, there are also some limitations to its use in this context. DNA degrades rapidly in the environment (Stricker et al. 2015) and therefore is only indicative of contemporary species presence. This constraint makes it possible to have a temporal mismatch between measured variables (species interactions) and processes of interest (gene flow). It is also possible that we are faced with a similar temporal mismatch with landscape data and gene flow in landscape genetics studies depending on the temporal scale of reproductive attributes of the focal species and that of landscape change. However, DNA can be preserved in sediments far longer than in exposed environments and provide a more historic view of community assemblages (Bálint et al. 2018). Future studies incorporating eDNA in landscape genetics may want to consider samples from sediments to avoid potential disparity of contemporary eDNA samples with measures of gene flow.

Combining local and landscape attributes to inform functional connectivity

Although we did not find strong support for both local and landscape characteristics influencing functional connectivity of *D. wrightorum*, our use of eDNA yielded additional interesting results such as the relationship between *D. wrightorum* abundance and patch-level vegetation. Combining information on both local and landscape processes in future landscape genetics studies allows for identifying drivers of functional genetic connectivity operating across scales. Among amphibians, both local and landscape features have been found to be major drivers of functional connectivity across several species and landscapes (Murphy et al. 2010; Watts et al. 2015; Robertson et al. 2018). Similar to our findings, other studies show that water, and particularly streams are important to functional connectivity of amphibians (Spear et al. 2005; Pilliod et al. 2015). Different measures of water availability were strong drivers of functional connectivity in all studies involving amphibians and the combination of local and landscape variables (Murphy et al. 2010; Watts et al. 2015; Robertson et al. 2018), though water availability may be important in different ways depending on the species and habitat.

Other studies using this approach to study functional connectivity of amphibians have found support for both local and landscape variables. For example, one study of boreal chorus frogs (*Pseudacris maculata*) in Colorado found that local and landscape measures of moisture availability, landscape topographic complexity, variation in annual precipitation, and underlying connectivity of wetlands were important for functional connectivity (Watts et al. 2015). Another study on sister taxa frogs (*Rana pretiosa* and *R. luteiventris*) in Oregon and Idaho found similar results regarding functional connectivity (Robertson et al. 2018). For both species, landscape measures of temperature and moisture had the most impact on functional connectivity, though there was variation among regions studied. In the central Oregon and Blue Mountain areas, both local hli and elevation, and metrics of landscape moisture and temperature were drivers of functional connectivity (Robertson et al. 2018). Finally, functional connectivity of *R. luteiventris* in Idaho was best explained by local measures of productivity and predation and landscape measures of temperature/moisture and topography (Murphy et al. 2010).

We also see a similar pattern of the importance of including both local and landscape variables to explain functional connectivity in other taxa. In a study involving northern Idaho ground squirrels (NIDGS; *Urocitellus brunneus*) and southern Idaho ground squirrels (SIDGS; *U. endemicus*), both local and landscape characteristics were influential, though variables important for functional connectivity differed between species (Zero et al. 2017). NIDGS functional connectivity was driven by local productivity (hli) and landscape topographic complexity (elevation relief ratio (err)), whereas local productivity (frost free period, hli, growing season precipitation), landscape topographic complexity, and barriers (err and impervious surfaces) were important for SIDGS. Additionally, functional connectivity among flowering dogwood (*Cornus florida*) was best explained by a combination of maternal plant (patch) characteristics and landscape attributes (Dileo et al. 2014). Both local characteristics (floral output, degree of canopy clumping over the maternal tree) and landscape characteristics (open canopy between maternal tree "patches", occurrence of deciduous canopy between maternal tree "patches") were important for functional connectivity of flowering dogwoods (Dileo et al. 2014).

Conservation and management significance

Understanding gene flow, and in turn population dynamics and functional connectivity on the landscape, is vital for species conservation initiatives in the face of stressors such as habitat loss (Hanski 1998). Habitat fragmentation or other disruptions in functional connectivity can alter dispersal dynamics and may lead to isolation and increased extinction risk of affected species (Marsh and Trenham 2000). Our analyses indicate that the functional connectivity of *D. wrightorum* is best explained by stream network distances between sites. Conservation action for this species currently occurs at the scale of ponds and pond-level restoration, which includes invasive species removal and manipulation of hydrology. However, management for the species may improve by additionally focusing on connecting the landscape between sites to improve the functional connectivity of *D. wrightorum*. Though we recognize the general difficulty of managing hydrology across the landscape, stream restoration or protection may help maintain connectivity among populations of this species as well as provide benefits to entire biotic communities that rely on scarce water in an arid landscape. However, stream systems may also provide pathways for non-native species in this system such as bullfrogs and crayfish, necessitating any restoration to also be coupled with invasive species monitoring and management to benefit native fauna. Additional considerations such as the correlation between *D. wrightorum* abundance and local vegetation that may help prioritize other demographic processes should also be considered to complement conservation action for gene flow and provide a holistic management plan for this species.

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