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Genetic approaches reveal a healthy population and an unexpectedly recent origin for an isolated desert spring fish

Brian L. Sidlauskas^{1*} , Samarth Mathur² , Hakan Aydoğan¹ , Fred R. Monzyk³ and Andrew N. Black⁴

Abstract

Foskett Spring in Oregon's desert harbors a historically threatened population of Western Speckled Dace (*Rhinichthys klamathensis*). Though recently delisted, the dace's recruitment depends upon regular removal of encroaching vegetation. Previous studies assumed that Foskett Dace separated from others in the Warner Valley about 10,000 years ago, thereby framing an enigma about the population's surprising ability to persist for so long in a tiny habitat easily overrun by plants. To investigate that persistence and the effectiveness of interventions to augment population size, we assessed genetic diversity among daces inhabiting Foskett Spring, a refuge at Dace Spring, and three nearby streams. Analysis revealed a robust effective population size (N_e) of nearly 5000 within Foskett Spring, though N_e in the Dace Spring refuge is just 10% of that value. Heterozygosity is slightly lower than expected based on random mating at all five sites, indicating mild inbreeding, but not at a level of concern. These results confirm the genetic health of Foskett Dace. Unexpectedly, genetic differentiation reveals closer similarity between Foskett Dace and a newly discovered population from Nevada's Coleman Creek than between Foskett Dace and dace elsewhere in Oregon. Demographic modeling inferred Coleman Creek as the ancestral source of Foskett Dace fewer than 1000 years ago, much more recently than previously suspected and possibly coincident with the arrival of large herbivores whose grazing may have maintained open water suitable for reproduction. These results solve the enigma of persistence by greatly shortening the duration over which Foskett Dace have inhabited their isolated spring.

Keywords Demography, Population genetics, Genotyping-by-sequencing, Endemism, Range-restricted species, Desert fishes

Introduction

The renowned ichthyologist Carl Hubbs opened a seminal work by observing pithily “where there is water, there are fishes” [1]. Indeed, fishes inhabit every imaginable aquatic habitat on our planet, from the deepest oceanic abyss [2] to barely moistened leaf litter [3], and from the freezing poles [4] to isolated pools in torrid deserts [5]. Scientists studying these last, arid habitats often marvel at the ability of pupfishes, daces, gobies, salamander-fishes, mosquitofishes, rainbowfishes and others to persist in some of the world's most marginal waters [6–12]. The surprising ubiquity of fishes in isolated desert systems invites exploration of their origins. How long ago

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did they reach their fragmented and distant homes, and by what means? How long have they endured there? Conservation biologists have also rightly raised concerns about the continued ability of these small populations of highly endemic species to persist in the face of changing landscape uses and a warming climate [13–15]. And indeed, several of the earliest fishes listed as imperiled under the US Endangered Species Act inhabit small waters in the vastness of America's arid west, such as the Devils Hole Pupfish (*Cyprinodon diabolis*), Desert Dace (*Eremichthys acros*), Owens River Pupfish (*Cyprinodon radiosus*) and Cui-ui (*Chasmistes cujus*) [16].

The origin, persistence and conservation of endemic snails, amphipods, plants and even planarians in desert springs have also attracted considerable attention [17–22]. These taxonomically disparate studies have confirmed that recent speciation and divergence contribute to the high endemism of desert waters [23–25], but also revealed that isolated springs can harbor relictual populations of ancient, formerly widespread lineages. For example, the isopod *Pheratomerus latipes*, a spring-dwelling endemic of the Lake Eyre region of South Australia, is a relict of a more broadly distributed lineage that diverged in the Miocene [26]. Stinking Lake Spring in Oregon's high desert harbors a relictual lineage of Speckled Dace (*Rhinichthys osculus*) that diverged at least 3 million years ago from the daces that now inhabit the surrounding Malheur watershed and may merit recognition as a distinct species [27]. And indeed, some inhabitants of aquatic desert ecosystems radiated recently from within relictual lineages, making their spring habitats simultaneous cradles and museums of diversity [28].

Given the risk of habitat alteration or the likelihood of small populations accumulating deleterious mutations through genetic drift, the persistence of lineages in desert springs over thousands to millions of years presents something of an enigma. Fervent debate has erupted over the age and origin of some iconic species, such as Nevada's Devils Hole Pupfish, *Cyprinodon*

diabolis, [29–31], which maintains such a tiny population size that demographic models suggest a high probability of stochastic extinction over timescales exceeding a few thousand years [29, 32]. An exceptional ability to tolerate inbreeding and a relatively recent divergence time may explain how that pupfish has so far evaded extinction [33], but these factors may not generalize to other desert spring taxa. For some, such as the endemic desert spring amphipods of South Australia, relatively high genetic diversity and surprisingly large populations may insulate them from inbreeding and the deleterious effects of drift [21]. Occasional dispersal and gene flow augments the genetic diversity of other desert endemics such as *Fonscochlea* snails [34] *Chlamydogobius* fishes [11, 35] and *Ochthebius* beetles [36]. But, even in cases where occasional dispersal augments the genetic diversity of small populations trapped in tiny habitats, the ability of these taxa to avoid local extinction from stochastic alteration to their habitat remains remarkable.

This study investigates the genetic health and persistence of Fosskett Spring Speckled Dace (Fig. 1, hereafter Fosskett Dace) an isolated Oregon population of *Rhinichthys klamathensis* that was listed federally as a threatened, undescribed subspecies of *R. osculus* between 1985 and 2019 [37, 38]. The ichthyologist Carl Bond assigned probable subspecies status to Fosskett Dace due to phenotypic differences from dace in the surrounding Warner Valley and because of its presumed isolation since the end of the late Pleistocene pluvial period Carl Bond, Oregon State University, pers. comm. 1990, cited in [39]. Subsequent phylogeographic and population genetic studies discovered that Fosskett Dace are identical or nearly identical to daces from elsewhere in the Warner Basin at maternally inherited mitochondrial loci [27, 40] and concluded that they do not merit subspecies status alone. However, Fosskett daces do differ enough in microsatellite allele frequencies to merit recognition as a distinct and significant population [27] within a subspecies of Western Speckled Dace inhabiting the entire Warner



Fig. 1 OS 22833, voucher BLS20–102, *Rhinichthys klamathensis goyatoka*, Fosskett Spring, Oregon, photographed in an immersion tank immediately after euthanization

Valley. Moyle et al. [41] recognized this subspecies formally as *Rhinichthys klamathensis goyatoka*.

Foskett Dace are endemic to a small, aquifer fed pool (Fig. 2, top) and an outflow stream that ends in a tule and cattail marsh on the shore of the normally dry Coleman lakebed [42]. That spring lies within the Coleman subbasin of the Warner Lakes basin, an endorheic basin located primarily in southeastern Oregon, though extending slightly into Nevada and California (Fig. 3). Previous studies [27, 40] assumed that these fish came to occupy their tiny habitat about 10,000 to 12,000 years ago, during the final desiccation of pluvial Lake Warner [43, 44].

The population size of Foskett Dace fluctuates substantially from year to year, with the availability of open water habitat seeming to drive much of the variation. Presumably, the dace require open water for effective

recruitment [45]. Though historical data record a maximum of 27,787 individuals in 1997, from 2005 to 2011 estimates from a Lincoln-Peterson closed-capture estimator have averaged around 10% of that value. The decline coincides with a gradual disappearance of open water that began in 1987 when the Bureau of Land Management fenced the spring and its outflow system to exclude cattle [46]. Census population size for Foskett Dace dropped to 1728 individuals in 2011 during a period of extensive aquatic vegetation growth throughout the entire system, with abundances in 2012 and 2016 just slightly above that minimum [46–48] assessments after 2011 used a Huggins closed-capture estimator. Population sizes rapidly increased in the years immediately following controlled burns or manual removal of aquatic vegetation to increase open water habitat, reaching a recent peak of 24,888 individuals in 2014 Fig. 4 [48]. Population sizes prior to the mid twentieth century are unknown, though the dependence of successful recruitment on the availability of open water habitat likely held.

If Foskett Dace have indeed remained isolated throughout the dry conditions of the last 10,000 years, then their long persistence despite pronounced volatility in their population size raises an enigma. Though the capture-recapture studies did not calculate effective population sizes, N_e in wild populations is typically much lower than census population size, and sometimes only a tenth of that value [49]. Because historical census population sizes for Foskett Dace have often fluctuated around a few thousand individuals, then effective population sizes have plausibly dipped below the 500 reproducing individuals that Franklin [50] proposed as necessary to maintain the evolutionary potential of a population, and almost certainly below Frankham et al.'s [51] revised threshold of $N_e > 1000$. The lowest census population sizes also fall short of Traill, Bradshaw, & Brook's [52] estimate for the minimum viable population size for animal species, which they calculated as 4,169 individuals through meta-analysis.

Given their tiny range, fluctuating population size, apparent dependence on open-water habitat for reproduction [45], and lack of an obvious migration pathway between Foskett Spring and the streams in the remainder of the Warner Valley for the last 10,000 to 12,000 years, how have they escaped extinction due to failed recruitment when vegetation overran their pool, or from the gradual erosion of genetic diversity? Does their population contain enough individuals of sufficient genetic diversity to insulate their lineage from effects of inbreeding and bottlenecks over thousands of generations? Are they lucky enough to have avoided failed recruitment despite encroaching vegetation? Did



Fig. 2 Collection sites for *Rhinichthys klamathensis goyatoka* at Foskett Spring, Oregon (top) and Coleman Creek, Nevada (bottom), as photographed by the authors

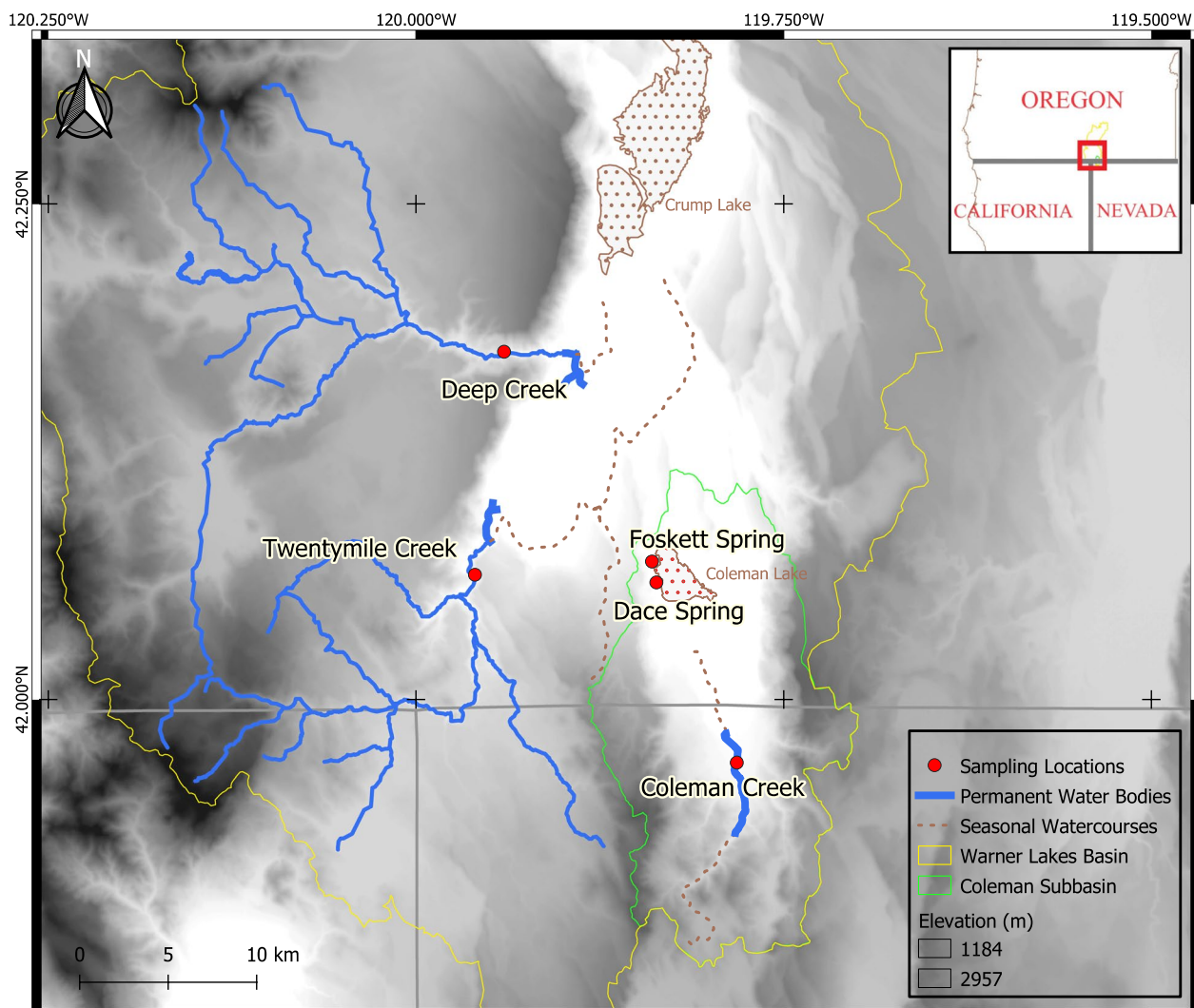


Fig. 3 Map of sampling locations at and near Foscett Spring. Blue waters (solid lines) are wet year-round, while dashed regions indicate water bodies that fill only occasionally. Foscett Spring, Dace Spring and Coleman Creek lie within the Coleman subbasin (green border) of the Warner Lakes basin (yellow border). Deep and Twentymile creeks lie outside the Coleman subbasin. Inset map shows the location of the southern Warner Valley near Oregon's borders with California and Nevada. The map was constructed in QGIS version 3.16.1 [119] using the river and stream network from USGS's National Hydrography Dataset [120] as downloaded from ArcGIS Data and Maps and NASA's SRTM Digital Elevation Data [121] as downloaded from USGS Earth Explorer at one arc-second resolution. Both datasets are freely available for redistribution as static electronic or printed maps with attribution to the data sources

an unknown factor keep the spring pool clear? Or, is the population in Foscett Spring simply younger than suspected?

This contribution harnesses modern molecular methods to quantify the effective population size and levels of genetic diversity among Foscett Dace, the refuge population at Dace Spring, and daces in three nearby streams, including a newly discovered and never-before-sequenced population in Nevada's Coleman Creek. By contributing a genetic assessment to

demographic monitoring, these objectives help maintain continued health and viability of the recently delisted Foscett Dace. We also evaluate the discreteness of Foscett Dace relative to the other populations and infer their demographic history and date of divergence. In so doing, we reveal Coleman Creek as the true ancestral source of Foscett Dace and solve the enigma of their purported persistence for 10,000 years in a small spring system that modernly becomes quickly engulfed by vegetation without human intervention.

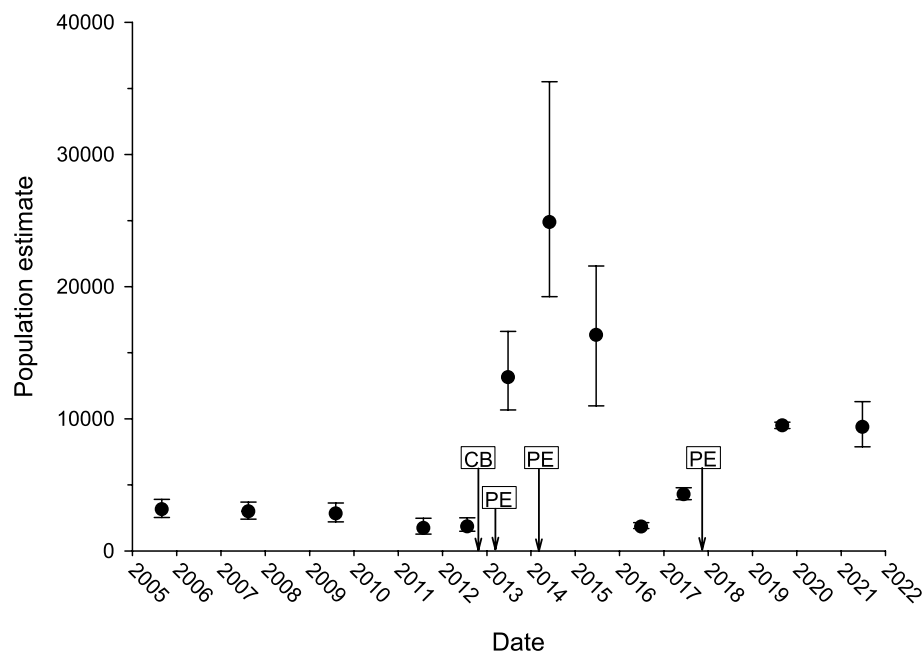


Fig. 4 Forkett Spring Speckled Dace population abundance from 2005 to 2021. Arrows on the date axis indicate habitat enhancements to increase the open-water habitat throughout the spring system and are offset by the year to indicate the month renovations occurred. CB=controlled burn, PE=pool excavation. Error bars are 95% confidence intervals. Data courtesy of the Oregon Department of Fish and Wildlife

Methods

Study area

The study examined specimens collected from five sites in two subbasins of the Warner Lakes basin. These subbasins connected hydrologically 12,000 years ago when Pleistocene Lake Warner filled the modern Warner Valley [44]. Forkett Spring, Dace Spring and Coleman Creek lie within the endorheic Coleman subbasin at the extreme southern end of the Warner Valley, while Twentymile and Deep Creeks lie outside the Coleman subbasin and drain towards Crump Lake (Fig. 3). Though no current hydrographic connection exists between these subbasins, a low sill just 1 meter in height separates them at their point of closest contact, and the basin floors differ by only eight meters of elevation [44]. Though the modern Coleman subbasin (Fig. 3, green border) does not currently connect hydrographically to the rest of the Warner Lakes basin, those small elevational differences imply that modest changes in water levels could re-establish connectivity.

Deep and Twentymile creeks represent the major perennial streams of the southern portion of the main Warner Valley. Each terminates in shallow marshlands on the valley floor during low discharge but during periods of high discharge, waters from the creeks inundate large portions of the valley floor and flow northward through sloughs and irrigation canals into the semi-permanent Crump Lake [53].

Within the Coleman subbasin, Forkett Spring (Fig. 2, top) originates on the western slope of the normally dry Coleman Lake (Fig. 3), which fills only during rare heavy rainfalls [54]. The spring forms a 33-m² pool that feeds a small outflow channel for approximately 95 m and terminates in a shallow marsh at the edge of the dry lakebed. Adult and juvenile Forkett Dace inhabit all the wetted portions of this spring system. Dace Spring is a smaller spring system located just to the south of Forkett Spring. Though historically lacking fish, Dace Spring was modified in 2010 to support a translocated refuge population of Forkett Dace. Across the Nevada border at the extreme southern end of the Coleman subbasin, Coleman Creek (Fig. 2, bottom) is a small intermittent stream perennially fed by small springs. Its short (~5 km) reach terminates in an irrigation reservoir except during periods of high discharge when flows can reach Coleman Lake, implying the possibility of fish migration between Coleman Creek and Forkett Spring through Coleman Lake during unusually wet years. Prior to this study, the population of dace in Coleman Creek was unknown to science.

Sample collection

Samples of adult daces that were used for genetic analyses were collected in 2020 from Coleman Creek ($N=48$), Forkett Spring ($N=48$), Dace Spring ($N=48$), Deep Creek ($N=20$) and Twentymile Creek ($N=26$) under Oregon Scientific Taking Permit 24,050 and license

39,884 issued by the Nevada Department of Wildlife. Fish were captured using minnow traps baited with sandwich bread and euthanized with tricaine methanesulfonate (MS - 222) at a concentration of 0.5 g/L in natural spring water, in accordance with Animal Care and Use Protocol 2020–0115 approved by Oregon State University's Institutional Animal Care and Use Committee (IACUC). Following euthanasia, selected specimens were photographed in an immersion tank (e.g., Fig. 1) and all specimens were immersed in ice water briefly while fin clips and plugs of epaxial muscle were collected. Voucher specimens for all tissue samples were collected and fixed in 10% formalin and have been accessioned into the Oregon State University Ichthyology Collection under catalog numbers OS22824, OS22831, OS22832, OS22833, OS22834, OS22835, OS22836, OS22837 and OS23148.

Library preparation and sequencing

DNA extraction, library construction, and sequencing were all conducted at Oregon State University's Center for Quantitative Life Sciences, largely following the methodology of Elshire et al. [55]. The main modification from this protocol involved the addition of a second restriction enzyme (see below). For each sample ($N = 190$), genomic DNA was isolated from ~50 mg of tissue using a DNeasy Blood and Tissue Kit (Qiagen), normalized to 20 ng/uL based upon Qubit fluorometer (Thermo Fisher Scientific) and fragmented using a double restriction digest with *pstI* and *mspI* on 200 ng DNA. Following restriction enzyme digest, Illumina sequencing adapters and barcodes were ligated to individually fragmented DNA sequences. PCR products were cleaned using QIAquick PCR purification kits (Qiagen) and quality was assessed on an Agilent 2100 Bioanalyzer and loading concentration determined by qPCR. Pooled samples were then sequenced across two lanes of an Illumina HiSeq 3000, using paired-end 151-bp sequence chemistry.

Quality filtering and SNP calling

Raw Illumina reads were assessed for quality using FASTQC (v0.11.9; Babraham Bioinformatics, Babraham Institute) and MULTIQC v1 [10, 56]. prior to assembling sequence fragments into putative genetic loci using STACKS v2 [52, 57]. The *process_radtags* script in STACKS was used to demultiplex raw sequences by assigning individual reads to corresponding samples through the unique combination of ligated in-line barcodes and standard Illumina read indices [55]. After removing the verified barcodes, truncated 141-base reads were checked for the presence of restriction cut-site sequences and only those reads containing correct motifs, or those with at most one base-call error (*-r* parameter), were retained. Using a sliding window of 21 bases (15% of read

length), reads that showed an average decrease in quality score (Q score < 10; 90% base-call accuracy) and those with any uncalled nucleotides were removed.

Demultiplexed reads were assembled into loci using the default parameters (but see below) of the STACKS *denovo_map.pl* wrapper program, which sequentially executes each core component of the pipeline. Retained loci were required to have a minimum allele depth of $5\times$ (*-m* 5), be present in $\geq 80\%$ of every population sample (*-min-samples-per-pop* 0.80) for all five populations (*-min-populations* 5) and have a minimum minor allele count of 3 (*-mac* 3). Furthermore, loci with an excessive number of reads (> 2 SD above the mean depth) were filtered to remove any potentially merged paralogous loci. To maintain independence of loci with multiple polymorphisms, only the first SNP from each locus was retained. Furthermore, to screen close relatives in population samples, which could bias results (e.g., estimates of effective population size), KING-ROBUST [58] was used to estimate the degree of relatedness between individuals using their kinship coefficient. The proposed cutoff values [58] were used to identify and remove one individual from each pair of 1st or 2nd degree relatives discovered.

Population genetic analyses

The filtered dataset was exported in PLINK file format [59] and converted to various file formats using PGD-SPIDER v.2.0.5.2 [60]. Effective population size (N_E) was estimated using the linkage disequilibrium method implemented in the software package NEESTIMATOR v.2.01 [61]; GENODIVE v.3.0 [62]; was used to calculate overall and per-locus observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding coefficients (F_{IS}). Overall and per-locus F_{ST} values [63] were calculated using DIVERSITY v.0.04–22 [64]; in R v.3.42 [65]. To visualize groups of genetically similar individuals, a principal component analysis (PCA) was conducted on a matrix of allele frequencies using the package ADEGENET v.2.01 [66]; in R. For the PCA, data were scaled and centered, and missing values were replaced with mean population values.

Population structure was evaluated by estimating individual admixture proportions using the Bayesian clustering algorithm implemented in ADMIXTURE v.1.30 [67];. Model complexity was determined for each value of K (1–8) using the cross-validation function (*-cv*) to identify the value of K with the lowest associated error. The most likely model was then selected, and Q-scores were plotted with GGLOT2 v. 3.0 [68]; for context, $\pm 1K$ are plotted. We created a population-level neighbor-joining (NJ) dendrogram based on pairwise weighted F_{ST} [63] differences between each population pair estimated using VCFTOOLS [69].

To test for historic admixture between different populations and to identify the likely origin of the dace population in Foscett Spring, we used the three-sample D-statistic (D_3) [70]. This D-statistic (also called the ABBA-BABA test) infers the post-divergence gene flow between divergent lineages based on gene genealogies [71] and can test for gene flow of alleles among three populations with no outgroup (i.e., without polarization of alleles as ancestral or derived). Briefly, D_3 measures discordance in branch lengths in tree topologies based on pairwise genetic distances between different samples. If there are three samples A, B, and C where A is more closely related to B than to C, and d_{A-C} is the genetic distance between A and C and d_{B-C} is the genetic distance between B and C. Given those definitions, D_3 can be calculated as:

$$D_3 = \frac{d_{B-C} - d_{A-C}}{d_{B-C} + d_{A-C}}$$

These discordance patterns should occur in equal frequencies between the two branches (A-C and B-C) and thus under expectation of no historic gene flow, $D_3 = 0$. Post-divergence gene flow between B and C would lead to $D_3 > 0$ and gene flow between A and C results in $D_3 < 0$. Since the D_3 statistic only requires a single sample from each population, we estimated genetic distances for each individual pair and estimated D_3 for all samples in each population trio in our dataset. We used D_3 estimates to identify the ancestral population of Foscett Dace by considering whether Coleman Creek or Deep Creek were the most likely source of gene flow into Foscett Spring, and therefore the probable ancestral population. These comparisons and the demographic model discussed below can only accommodate trios, not quartets, and so we chose Deep Creek as the exemplar locality from the population inhabiting the Warner Valley outside the Coleman subbasin (see results). Pairwise individual genetic distances were calculated using TASSEL [72] and D_3 for each population trio was calculated using R [65]. We estimated the mean and 95% CI from the distribution of D_3 estimates for each population trio using the Z-distribution. We did not use the classic D-statistic because it estimates patterns of gene flow of a derived allele using an ancestral outgroup, and in this case the outgroup designation was uncertain because the order of colonization among the five localities was unknown.

Reconstructing demography of Western speckled dace populations

We reconstructed demographic history from the joint site frequency spectra (SFS) data with GADMA v. 2.0.0rc20 [73] using the diffusion approximation as implemented in $\partial a \partial i$ [74]. This program estimates the population size

(N_e) changes, divergence times (T), and migration rates (m) among two or three populations. Given the limit of three populations, we modeled history using dace from Foscett Spring, Coleman Creek, and Deep Creek, each representing one of the three major populations identified by the PCA and Admixture analysis. Though Twentymile Creek dace are genetically similar to Deep Creek dace (see results), they are not identical. We did not combine these subpopulations into a single population in the demographic models because this would have introduced metapopulation dynamics and subpopulation gene flow, which could render the results unreliable. We excluded the samples from Dace Spring because this is an artificial subpopulation with a known date of translocation.

For each demographic model, GADMA simulates the joint SFS and compares it to the observed SFS from population genetic data using log-likelihoods. It then chooses the most likely model by comparing AIC scores. To estimate confidence intervals (CI) of the parameter estimates (N_e , T , m) for the most likely demographic model, we bootstrapped the observed joint SFS 100 times using independent subsets of SNPs. We converted genotype data to get observed SFS using EASY-SFS (<https://github.com/isaacovercast/easySFS>). We conducted demographic model simulations under fast and slow mutation rates. In the fast mutation scenario, we used a point mutation rate of $\mu = 6.6 \times 10^{-8}$, which is where Martin and Höhna [31] centered the prior in their analysis of Devils Hole Pupfish, and which Recknagel et al. [75] measured as the actual mutation rate for RADSeq loci in the Midas cichlid (*Amphilophus* spp.). For the slow mutation scenario, we used a point mutation rate of $\mu = 1.2 \times 10^{-8}$ following Chafin et al.'s [76] analysis of the *Gila robusta* species complex, another desert-dwelling lineage within Leuciscidae. Though they did not specify the source of their mutation rate estimate, we note that this slower rate more closely aligns with other recent estimates for a phylogenetically diverse group of vertebrates [77].

Speckled Dace rarely live for more than 3 years and never beyond four, and they typically reproduce at the age of two, though males may mature a year earlier than females [78–80]. Given the available information on life history, we set the generation time to two years, keeping all other parameters to default values.

Results

Quality filtering and SNP calling

Using 2×151 sequencing chemistry, the Illumina HiSeq 3000 yielded 758M reads across two sequencing lanes. Barcode sequences found within each multiplexed *fastq* file allowed assignment of paired-end reads to individual samples using the *process_radtags* script in STACKS. This resulted in 656M retained sequences (87.5%) after

Table 1 Effective population size estimates for the five sampled populations, calculated using the linkage disequilibrium method as implemented in NeEstimator (V.2)

	N_e	Lower	Upper
Coleman Creek	1,418	1,193	1,744
Dace Spring	506	472	546
Foskett Spring	4,950	2,937	15,619
Deep Creek	Infinite	–	–
Twentymile Creek	Infinite	–	–

*Based upon minor allele frequency cutoff of ≥ 0.01

Table 2 Population genetic metrics for the five Western Speckled Dace populations prior to removing relatives. Mean observed (H_o) and expected (H_e) heterozygosity, and mean inbreeding coefficient (F_{IS})

	H_o	H_e	F_{IS}
Coleman Creek	0.163	0.172	0.053
Dace Spring	0.156	0.163	0.038
Deep Creek	0.165	0.175	0.056
Foskett Spring	0.158	0.164	0.036
Twentymile Creek	0.171	0.181	0.057

accounting for reads missing barcodes, restriction enzyme cut sites, or those exhibiting low base call quality. Following de novo assembly of loci among 190 individuals, samples had a mean depth of coverage (among 0.2 M loci) of 134x. Among these genotyped loci, there remained 4,317 loci that met the sample (80%) / population (5) constraints. After removing two individuals that had a low genotyping rate (<70%) and eight individuals representing part of a pair of 1st or 2nd degree relatives, there remained 180 samples genotyped at 3,354 SNPs.

Population genetic analyses

Using the linkage disequilibrium method, effective population sizes were moderately low for Dace Spring ($N_e = 506$), high for Coleman Creek ($N_e = 1,418$) and Foskett Spring ($N_e = 4,950$) and infinite for the Deep and Twentymile creek populations (Table 1). To assess the genetic health of the populations, we calculated

overall and per-locus observed heterozygosity (H_o), expected heterozygosity (H_e) and inbreeding coefficients (F_{IS}). All five populations showed similar levels of H_o (0.156–0.171) while the creek populations (0.175–0.181) exhibited marginally higher levels of H_e than the spring populations (0.163–0.164; Table 2). The close relationship between expected and observed diversity resulted in very low F_{IS} values among all Western Speckled Dace populations (0.036–0.057; Table 2). Pairwise F_{ST} estimates (Table 3) illustrated that Foskett Spring and Dace Spring daces have highly similar gene pools ($F_{ST} = 0.0055$), which is not surprising given that Dace Spring lacked fish until ~100 daces were transplanted there from Foskett Spring in 2010 and 2011. Twentymile Creek daces differ moderately from those inhabiting Deep Creek ($F_{ST} = 0.0604$). The comparisons involving Coleman Creek showed moderate genetic differentiation from the two spring populations ($F_{ST} = 0.0671$ –0.0704), and twice that difference from the two creek populations outside of the Coleman subbasin ($F_{ST} = 0.1128$ –0.1275). Thus, daces in Foskett Spring are genetically more like Coleman Creek daces than they are like Twentymile or Deep Creek daces ($F_{ST} = 0.1467$ –0.1605).

PCA of allele frequencies clustered the individuals from Foskett and Dace springs together, united individuals from Twentymile and Deep creeks, and separated Coleman Creek dace cleanly from the other clusters (Fig. 5). PC1 (explaining 9.4% of variance) separates all three clusters, though it places Coleman Creek dace closer to the individuals from the spring populations than the other creeks. PC2 (3.6% of variance) clearly captured the variation separating Coleman Creek from all other populations (Fig. 5). Cross validation of K 2–8 identified K = 3 as the having the lowest associated error. This model clearly clustered the two spring populations (Foskett and Dace) together, placed Coleman Creek dace in their own cluster, and grouped dace from Deep and Twentymile creeks (Fig. 6A). While associated error for the hypothesis of K = 4 exceeded that for K = 3, the clean separation of dace from Deep and Twentymile creeks under the four-group model implies subpopulation-level divergence between those two sites in the main Warner subbasin. Samples from Coleman Creek separated clearly from the other

Table 3 Pairwise global F_{ST} values among the five sampled Western Speckled Dace populations

	Coleman Creek	Dace Spring	Deep Creek	Foskett Spring
Coleman Creek				
Dace Spring	0.0704			
Deep Creek	0.1275	0.1644		
Foskett Spring	0.0671	0.0055	0.1605	
Twentymile Creek	0.1128	0.1499	0.0604	0.1467

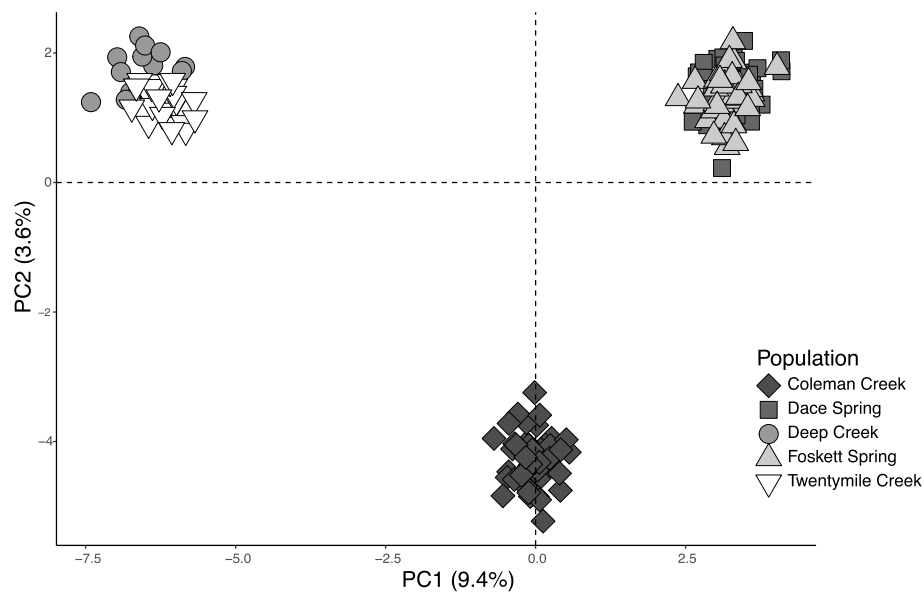


Fig. 5 Principal Component Analysis of Western Speckled Dace in the southern Warner Valley. First two axes (PC1 & PC2) generated from allele frequencies among the five sampled dace populations

groups assuming $K=3$ or 4, albeit with low to moderate admixture with the spring samples for $K=2-4$ (Fig. 6A). Coleman Creek is the only population to return a dominant signal of admixture at $K=2$. A neighbor joining dendrogram constructed from a weighted F_{ST} matrix of all pairwise populations concurs with the intermediacy of Coleman Creek dace relative to the Foscett and Dace springs fish on one hand, and the fish from Twentymile and Deep creeks on the other (Fig. 6C).

To explore historic admixture (i.e., gene flow) among the three geographically and genetically separate populations, we estimated the D_3 -statistic for a trio of localities (Foscett Spring, Coleman Creek, Deep Creek) representing the three major genetic clusters. We considered scenarios with either (a) Foscett Spring and Deep Creek most closely related, or (b) Foscett Spring and Coleman Creek most closely related. If the first scenario is correct, we would expect no gene flow between Coleman Creek and Foscett Dace ($D_3=0$), yet we uncover strong evidence of gene flow between the Coleman Creek and Foscett Dace populations ($D_3=0.054$; 95% CI=[0.0527–0.0558]) (Fig. 7A). This suggests that the scenario of an initial split separating the Coleman Creek population from a lineage leading to the Foscett and Deep Creek populations is incorrect. On the other hand, D_3 values more closely match predictions if we consider scenarios in which Deep Spring is ancestral and the Foscett Spring and Coleman Creek populations diverged more recently (Fig. 7B–C). In a scenario that considers Deep Creek to be most ancestral and postulates that Foscett Spring dace

diverged from the Coleman Creek population (Fig. 7B), we find no evidence of historic gene flow between Foscett Spring and Deep Creek populations and weak evidence of historic post-divergence gene flow between Coleman Creek and Deep Creek populations ($D_3=-0.005$; 95% CI=[−0.0066, −0.0033]). Similar weak evidence of historic gene flow between Coleman Creek and Deep Creek obtains if we consider Foscett Spring to be ancestral to the Coleman Creek population (Fig. 7C; $D_3=0.0046$; 95% CI=[0.0030, 0.0062]). Taken together, these results indicate that the deepest split occurred between the Deep Creek population and the lineage leading to the Coleman Creek and Foscett Spring populations. Historic gene flow between Coleman Creek and Deep Creek may then have occurred after the split between the Coleman Creek and Foscett Spring populations.

The ancestors of Coleman Creek dace founded the Foscett Spring population

We estimated divergence times and long-term N_e from three-population demographic models constructed under fast and slow mutation rate scenarios. These models simulate the joint site frequency spectra of the Western Speckled Dace inhabiting three localities (Coleman Creek, Deep Creek, Foscett Spring), representing the three modern populations. Table 4 lists the parameter estimates from the two models and Fig. 8 visualizes them.

Outputs from both models indicate that the modern diversity of Western Speckled Dace in the Warner Valley derives from two ancestral lineages (P_{11} and P_{12} in Fig. 8),

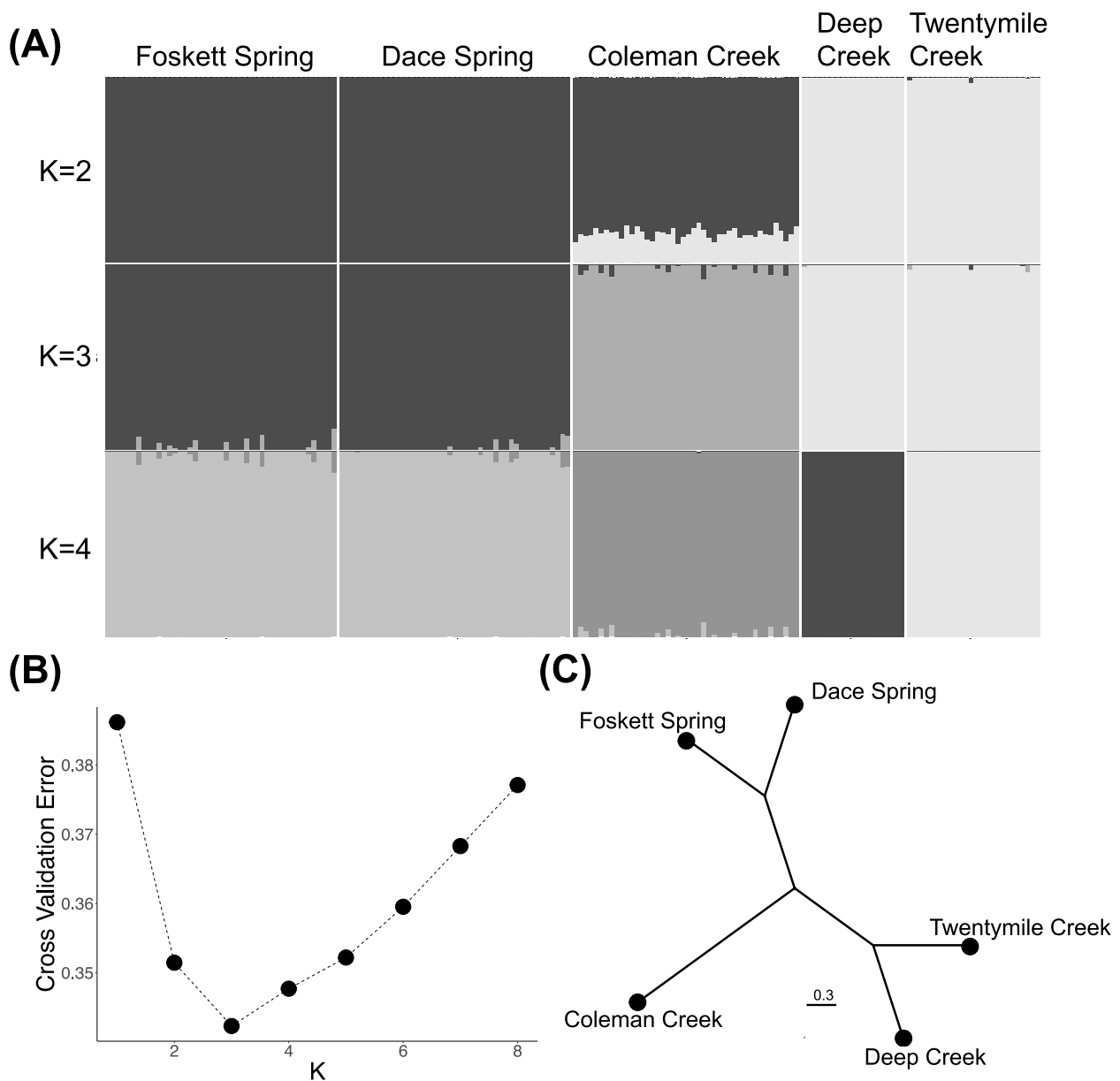


Fig. 6 Population structure of Western Speckled Dace in the southern Warner Valley. **A** Population structure plot showing ancestry proportions for each individual (Coleman Creek=44, Dace Spring=45, Deep Creek=20, Foscett Spring=45, Twentymile Creek=26) at different numbers of ancestral populations (K). The model identified $K=3$ as the most likely number of ancestral populations **B** Cross validation errors for all tested values of K in the population structure analysis shown in panel A. **C** Neighbor joining dendrogram constructed from a weighted F_{ST} matrix of all pairwise populations

each confined to a different subbasin of the Warner Valley. Less than a millennium ago, a portion of the Coleman subbasin lineage (P_{12}) founded the population in Foscett Spring while the lineage in the other subbasin (P_{11}) experienced a concurrent demographic contraction, possibly representing isolation of the Twentymile and Deep Creek subpopulations. Both fast and slow mutation models estimate quite recent colonization of Foscett Spring, with the

fast model estimating the divergence time between Foscett Spring and Coleman Creek 588 years ago [95% CI=572–595] and the slow model indicating divergence 896 years ago [95% CI=812–980]. The models differ more in their divergence time estimates between the two subbasins. The fast mutation rate model estimated that event at 3,784 years ago [95% CI=3712 – 3878] while the slow mutation rate model estimated 10,529 years [95% CI=9,989 – 11,069].

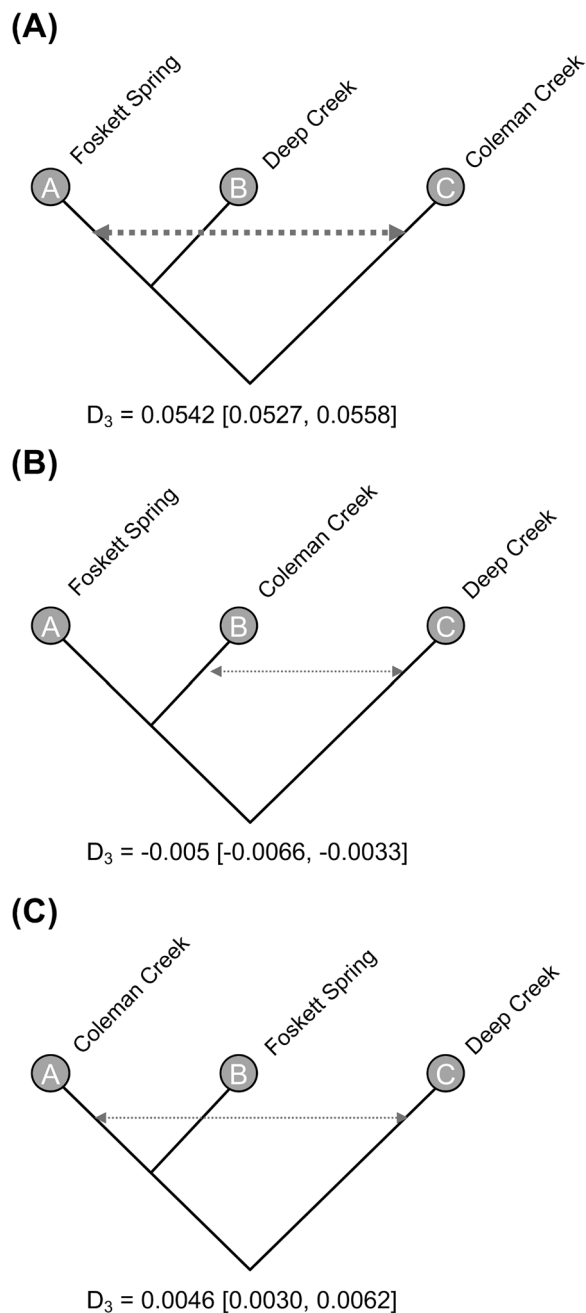


Fig. 7 Inference of historic admixture between Western Speckled Dace populations. We tested for evidence of historic gene flow between diverging populations by measuring the D_3 -statistic among population trios. In each case, population A is more closely related to B than to C. D_3 measures the discordance in branch lengths with $D_3 = 0$ means no gene flow, $D_3 > 0$ indicates gene flow between A and C, whereas $D_3 < 0$ represents gene flow between B and C. We estimated D_3 statistics for tree topologies where **A** Coleman Creek was sister to Foskett Spring and Deep Creek and **B-C** Deep Creek was sister to Foskett Spring and Coleman Creek. We find strong evidence of gene flow (shown by arrows) between Foskett Dace and Coleman Creek (A) and weak evidence of gene flow between Coleman Creek and Deep Creek (B-C). Numbers below each figure indicate mean D_3 for the given topology with 95% CI around the mean given in brackets. Thickness of arrows represents strength of gene flow. These results indicate that Foskett Dace likely diverged most recently from the Coleman Creek population, and that the Coleman Creek population experienced post-divergence gene flow with Deep Creek, possibly via secondary contact

Both models estimate robust effective population sizes for the founding lineage of dace in the Warner Valley ($N_{anc} = 1,890$ or $3,806$) and infer a roughly equal split of that population into the Coleman and main Warner subbasins. Thereafter, both models infer a stable effective population size ($P_{12,0} = P_{12,1}$) of just over 1,000 individuals in the Coleman subbasin. The models differ in their demographic reconstruction outside the Coleman subbasin, with the slow mutation rate model inferring a stable effective population size of 7,272 individuals in the main Warner subbasin (P_{11}) over nearly 10,000 years, and the fast mutation rate model inferring linear growth from 879 ($P_{11,0}$) to 5,863 ($P_{11,1}$) individuals over about three millennia. In either scenario, the population within the main Warner subbasin ($P_{11,1}$) was substantially larger than that in Coleman subbasin ($P_{11,2}$) until the event 588 or 896 years ago that restructured both lineages. At that time, both models concur that the P_{11} population (leading to the modern Deep Creek subpopulation of the population in the main Warner Valley, and presumably also the subpopulation in Twentymile Creek) underwent a strong bottleneck, while P_{12} split into the Coleman Creek population and Foskett Spring population, with most (88.7% or 84.7%) of these P_{12} ancestors remaining in Coleman Creek.

The dynamics of the three modern populations have differed over the most recent centuries. Though it was founded by relatively few (11.3% or 15.3%) of the Coleman valley ancestors, the contemporary Foskett Spring population grew exponentially from that small start ($FS_0 = 114$ or 198) to its current sizes ($FS_1 = 1,076$ or $1,958$) whereas the contemporary Coleman Creek population remained stable ($CC_0 = CC_1 = 955$ or $1,739$) after separating from the ancestral population. Since the

Table 4 Maximum likelihood parameters for three-population demographic model for Western Speckled Dace inhabiting Coleman Creek, Deep Creek, and Foscett Spring inferred using GADMA under fast and slow mutation rates. Values within brackets represent 95% confidence intervals estimated by bootstrapping observed site frequency spectra 100 times

Fast Mutation rate ($\mu = 6.6 \times 10^{-8} \text{ bp}^{-1} \text{ gen}^{-1}$)		Slow Mutation rate ($\mu = 1.2 \times 10^{-8} \text{ bp}^{-1} \text{ gen}^{-1}$)	
Log likelihood	– 9,239.14	Log likelihood	– 9,496.83
Population size [95% CI]		Population size [95% CI]	
NA_0	1,890 [1,789 – 1,923]	NA_0	3,806 [3,611 – 4,001]
$P11_0$	879 [866–884]	$P11_0 (= P11_i)$	7,272 [7,053 – 7,491]
$P11_i$	5,863 [5,813 – 5,901]		
$P12_0 (= P12_i)$	1,011 [998–1094]	$P12_0 (= P12_i)$	1,291 [1,082 – 1,500]
FS_0	114 [105–125]	FS_0	198 [167–229]
FS_i	1,076 [1,023 – 1,088]	FS_i	1,958 [1,637 – 2,279]
$CC_0 (= CC_i)$	955 [934–975]	$CC_0 (= CC_i)$	1,739 [1,561 – 1,917]
$DC_0 (= DC_i)$	1,136 [1,112 – 1,152]	$DC_0 (= DC_i)$	2,086 [1,812 – 2,360]
Migration rate per generation [95% CI]		Migration rate per generation [95% CI]	
$m_{p11-p12}$	1.1×10^{-2} [1.0 – 1.1×10^{-2}]	$m_{p11-p12}$	2.0×10^{-3} [1.8 – 2.2×10^{-3}]
m_{DC-CC}	8.0×10^{-3} [8.0 – 8.1×10^{-3}]	m_{DC-CC}	1.0×10^{-3} [0.9 – 1.1×10^{-3}]
m_{CC-DC}	4.0×10^{-3} [4.0 – 4.0×10^{-3}]	m_{CC-DC}	2.2×10^{-4} [2.1 – 2.3×10^{-4}]
m_{DC-FS}	3.1×10^{-3} [3.0 – 3.1×10^{-3}]	m_{DC-FS}	0
m_{FS-DC}	0	m_{FS-DC}	0
m_{CC-FS}	2.7×10^{-2} [2.6 – 2.7×10^{-2}]	m_{CC-FS}	2.0×10^{-3} [1.9 – 2.1×10^{-3}]
m_{FS-CC}	1.6×10^{-2} [1.6 – 1.6×10^{-2}]	m_{FS-CC}	0
Divergence time in years [95% CI]		Divergence time in years [95% CI]	
T1	3,783.8 [3,712.4 – 3,877.9]	T1	10,529 [9,989 – 11,069]
T2	587.5 [572.4–595.1]	T2	896 [812–980]
Population split ratio [95% CI]		Population split ratio [95% CI]	
Sp11	46.5% [44.7–48.3%]	Sp11	55.5% [53.7–57.3%]
SpCC	88.7% [85.1–92.3%]	SpCC	84.7% [82.5–86.9%]

NA_0 : size of ancestral population; $P11_0$: size of P11 immediately after divergence from ancestral population; $P11_i$: size of P11 immediately before second demographic split; $P12_0$: size of P12 immediately after divergence from ancestral population; FS_0 : size of Foscett Spring population immediately after divergence from P12; FS_i : size of Foscett Spring population after exponential expansion; DC_0 : size of Deep Creek population immediately after bottleneck from P11; CC_0 : size of Coleman Creek population after divergence from P12; m_{ab} : migration rate from population a to b; T_1 : divergence time between P11 and P12; T_2 : divergence time between Foscett Spring and Coleman Creek population

historic event that reduced effective population size circa 588 or 896 years ago, the contemporary Deep Creek sub-population has remained stable ($N_{DC} = 1,136$ or 2,086).

Our model inferred gene flow occurring at several points over the last few millennia. Unidirectional historic gene flow from the main Warner subbasin to the Coleman sub-basin ($m_{p11-p12} = 1.1 \times 10^{-2}$ or 2.0×10^{-3}) occurred between their initial subdivision and the event that founded the Foscett population. More recently, the demographic models inferred unequal and unidirectional gene flow among the three contemporary populations. Both models identified gene flow from Deep Creek into Coleman Creek ($m_{DC-CC} = 8.0 \times 10^{-3}$ or 1.0×10^{-3}) and the fast mutation rate model also inferred low flow from Deep Creek to Foscett Spring ($m_{DC-FS} = 3.1 \times 10^{-3}$). Low levels of gene flow from Coleman Creek to Deep Creek were also identified ($m_{CC-DC} = 4.00 \times 10^{-3}$ or 2.2×10^{-4}) but neither demographic model estimated any gene flow from Foscett Spring to

Deep Creek ($m_{FS-DC} = 0$). The highest migration rate was identified from Coleman Creek to Foscett Spring ($m_{CC-FS} = 2.7 \times 10^{-2}$ or 2.0×10^{-3}). The fast mutation rate model also inferred limited flow from Foscett Spring to Coleman Creek ($m_{FS-CC} = 1.6 \times 10^{-2}$).

Discussion

Three distinct populations of Western Speckled Dace inhabit the southern Warner Valley

All our population genetic results clearly indicate three distinct clusters of Western Speckled Dace, each confined to a distinct hydrographic system within the southern Warner Valley (Figs. 5 and 6A). Analysis of genome-wide SNPs from GBS readily distinguished Foscett Spring dace from conspecifics collected from Twentymile or Deep creeks, confirming the results from an earlier microsatellite study [27]. Fish from the newly discovered locality in Coleman Creek form a third cluster that was readily

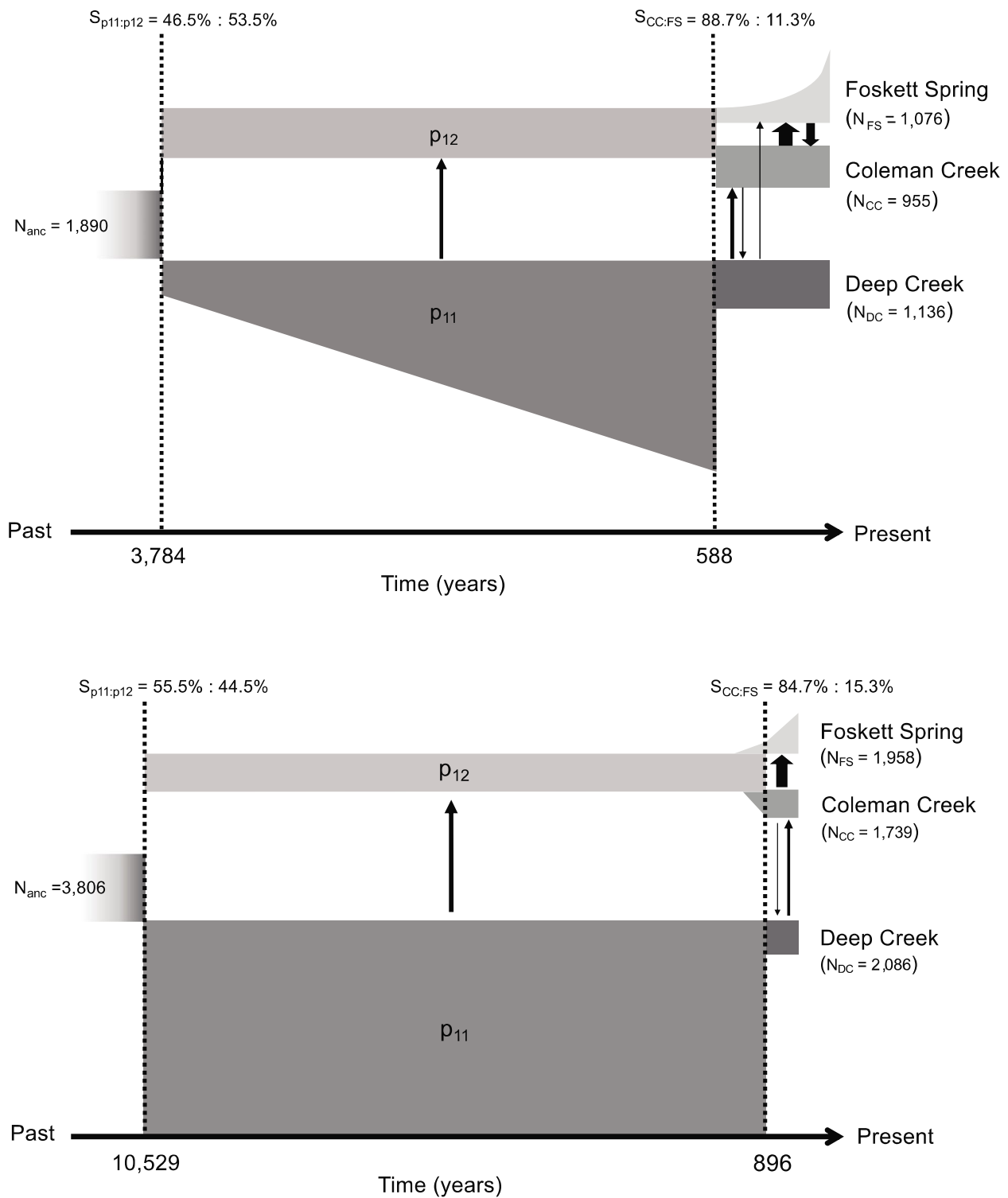


Fig. 8 The most likely three-population demographic models for Western Speckled Dace in the southern Warner Valley, constructed by simulating joint site frequency spectrum data using GADMA under high ($\mu = 6.6 \times 10^{-8}$) and low ($\mu = 1.2 \times 10^{-8}$) mutation rates and a generation time of two years. Mean N_e and divergence time estimates are presented here; all parameters and 95% confidence intervals around mean estimates are provided in Table 4. Arrows represent direction of post-divergence migration (gene flow) among the populations, with thickness indicating the magnitude of the migration rate. Bison arrived in the southern Warner Valley between 642 to 257 years ago, coincident with or just after the demographic shifts in dace populations inferred at 588 or 896 years before present

distinguishable from the other groups in all analyses. As expected, given their origin as a recent translocation, fish in Dace Spring are not distinguishable from those in Foscett Spring.

Fish from the two stream localities outside the Coleman subbasin cluster separately in neighbor joining dendrograms and in an Admixture analysis with $K=4$ (Fig. 6). However, $K=4$ is only the second most likely model, and the most likely model ($K=3$) groups dace from Twentymile and Deep creeks into a single population. We interpret these patterns as reflecting separate, recently diverged subpopulations within the population that occupies the Warner Lakes basin outside of the Coleman subbasin. Wiesenfeld et al. [81] obtained similar results from Western Speckled Dace within California's Klamath-Trinity basin, where they discovered three genetic clusters despite the lack of any obvious physical barrier separating two of them. If this kind of substructure within river systems is typical for *Rhinichthys klamathensis*, it might indicate that individuals tend towards strong site fidelity. Alternatively, sampling at more sites within the Twentymile and Deep stream networks might reveal intermediate individuals that blur the genetic distinction.

Overall, these results parallel other recent studies of *Rhinichthys* by recovering a genetically distinct population in each isolated drainage [12, 82]. Similar results have been obtained in many other fishes inhabiting desert springs [10, 83], dendritic riverscapes [84–86], or both [87]. Both situations create landscapes that can restrict gene flow, either by stranding fishes in isolated pools and springs, or by separating headwater populations by many river miles [13]. At the same time, it is worth noting that not every desert spring necessarily harbors fishes representing a distinct population. For example, Campbell et al. [25] found just three distinct genetic clusters among 10 relicual spring localities in their study of *Crenichthys baileyi* in southeastern Nevada. Thus, while spring populations do often represent important components of genetic diversity, their distinctiveness must be tested as Stockwell et al. [88] did in their study of spring-dwelling populations of *Cyprinodon tularosa*, not assumed.

Foscett dace are genetically healthy and stable

Despite their isolation and tiny habitat, Foscett Dace appear to be doing remarkably well. We estimated their effective population size at 4,950 fish, which triples the value for the probable source population in Coleman Creek (Table 1), exceeds Frankham et al.'s [51] threshold of $N_e > 1000$ to maintain population viability over evolutionary time, and surpasses the median value for minimum viable population size in animals (4,169) calculated

in a meta-analysis of three decades worth of case studies [52]. The large N_e for Foscett Spring based on the samples collected in 2020 reconciles well with the 2019 demographic estimate of 7,354 (95% CI 6975–7833) 'adult-sized' dace (fish > 35 mm fork length) using a mark-recapture method [38], particularly given that Speckled Dace do not begin to reproduce until they reach around 60 mm [78]. These numbers are substantially higher than some historical estimates, which have dipped below 2,000 individuals in years of vegetation encroachment, as they did in 2011 [46, 47]. Provided that the current population size remains stable, there seems to be little chance of stochastic extinction of Foscett Dace in the near future.

Our estimates of long-term and contemporary N_e suggest that Foscett Spring dace populations have expanded demographically in the last few generations. Long-term N_e is defined as the harmonic mean of per-generation N_e of a population over evolutionary time (hundreds of generations), whereas contemporary N_e measures population sizes within the previous generation or over just a few [89]. While periodic connectivity with the Coleman Creek population during wet years could help explain the population expansion within Foscett Spring over evolutionary time (see below), the high contemporary N_e of Foscett Dace most likely reflects the active restoration work to increase open water habitat at Foscett Spring [38, 90].

Whatever their cause, the high estimates for N_e in Foscett Spring help to explain the low inbreeding coefficient (0.036, Table 2). While not zero, the very slight amount of inbreeding certainly represents no cause for concern. This number is nearly zero, and lower than the estimates for the even larger populations in Twentymile and Deep creeks, suggesting that the Foscett Spring population is at least as healthy as those in the stream networks. Indeed, they are arguably faring better than the stream fishes, perhaps because of the lack of any competitors or predators in their habitat. Foscett Dace enjoy sole occupancy of their spring, while dace in Twentymile and Deep creeks share their habitat with several other fishes, including suckers, tui chubs, trouts, and introduced centrarchids. The first two of these plausibly compete with dace, while the latter two certainly eat them.

As for the refuge population at Dace Spring, the estimated N_e of 506 individuals is just a tenth of the value in Foscett Spring, which is perhaps not surprising given that only ~ 100 fishes founded the subpopulation at this locality a decade ago. This value of N_e is relatively low, and while not at the extremes of 122 individuals that Tian et al. [33] calculated for *Cyprinodon diabolis*, or the 28 individuals that Black et al. [91] estimated for *Cyprinodon bovinus*, it may be low enough to incur some risk

of stochastic extinction over dozens or hundreds of generations.

Finally, the consistent pattern of reduced observed heterozygosity compared to expected heterozygosity does suggest that allele dropout (due to polymorphisms in restriction enzyme cutsites) may have influenced diversity estimates for all population [92]. If so, the diversity estimates presented herein provide a conservative assessment of population heterozygosity as the true variation could be slightly higher than estimated.

Recency of origin and intermittent connectivity help to explain the persistence of Fosskett Dace

In the introduction, we raised the question of whether luck, genetic diversity, or youth explains the persistence of Fosskett Spring Speckled Dace in their tiny habitat. Analyses discussed above indicate that many breeding adults inhabit Fosskett Spring, and that these resemble their nearby stream-dwelling cousins in heterozygosity and in their low levels of inbreeding. Those findings indicate that genetic diversity may have played a role in insulating the population from a stochastic drift towards extinction. That said, results from the demographic analysis indicate that genetic diversity does not provide the whole story. Fosskett Dace are also much younger and less isolated than previously suspected.

Our demographic model indicates that Western Speckled Dace colonized Fosskett Spring from Coleman Creek within the last 500–1000 years, not 10,000 years ago during the desiccation of pluvial Lake Warner. That temporal restriction greatly increases the plausibility of the dace's persistence because it implies that only 250 to 500 generations have passed since isolation, not 5,000. Given that the long-term N_e of Fosskett Dace could be as low as 1,076 (Table 4, Fig. 8, fast mutation rate scenario), 5,000 generations could have exceeded the number needed for alleles to drift to fixation (4304, calculated as $4N_e$ following [93]), but 250 or 500 generations fall far short of that threshold. The reduced duration of persistence also implies far fewer opportunities for rare catastrophic events to cause population crashes. In other words, the danger of drift and the odds of survival are much better over the short term than the long term. Therefore, the need for luck diminishes, and the enigma of persistence largely disappears when the question reframes on the scale of centuries rather than millennia.

The demographic results also suggest that periodic gene flow may have helped to insulate Fosskett Dace from the effects of drift (Figs. 7 and 8). The D_3 statistics and demographic models both infer high levels of historic gene flow between Coleman Creek and Fosskett Spring after their divergence. The D_3 statistics also show weak evidence of gene flow between the Coleman Creek and

Deep Creek populations. The GADMA models concurred, adding the insight of unequal rates of migration. Gene flow from Deep Creek into Coleman Creek was two to five times as prevalent as the reverse (Table 4), and unidirectional gene flow from Deep Creek into Fosskett Spring may have also occurred (Fig. 8, fast mutation rate scenario). Overall, these results help reconstruct how the Fosskett Spring population overcame a strong founding bottleneck, in which their originators represented just ~10% or ~15% of the ancestral population in the Coleman subbasin. Once the spring population established, it grew exponentially (Fig. 8), likely through a combination of recruitment and a periodic influx of migrants from Coleman Creek population whenever it flooded the dry Coleman lakebed.

By virtue of these results, the daces of the Warner Valley join a growing list of case studies illustrating how small populations in river systems can persist over evolutionary time by maintaining population sizes and genetic diversity through periodic connectivity [11, 34, 36, 94, 95]. They reinforce Mossop et al.'s [35] conclusion that ephemeral waters represent important dispersal routes in arid ecosystems. Such connections, however fleeting, seem to help freshwater fishes in isolated waters to overcome the small population sizes and low genetic diversity [96–99] that could otherwise promote a drift towards imperilment or extinction.

Either reconstructed demography accords with hydrologic history

The slow mutation rate model (Fig. 8, lower panel) estimated an initial divergence 10,529 years ago between the Coleman subbasin and the rest of the Warner Lakes basin. That date accords closely with the end of the Pleistocene pluvial period around 10,000 years ago [43, 100] and agrees with Wriston and Smith's [44] archaeological conclusion that pluvial Lake Warner ceased filling the Warner Valley 10,300 years ago. This close agreement between the genetic age of the initial population split and the onset of xeric conditions lends substantial credence to the slow mutation rate scenario and may indicate that these older dates represent the more likely reconstruction.

The fast mutation rate model estimated an unexpectedly recent split between the subbasins at 3,784 years ago, well after the desiccation of pluvial Lake Warner. At first glance, the recency of that date makes the fast mutation rate scenario seem less plausible. However, this divergence estimate aligns with the end of a "Neopluvial" period with higher than present-day precipitation between 5,000 and 3,000 years ago [101, 102]. The paleobotanical record at the geographically proximate Lily Lake site records a noted spike in precipitation about

3,300 years ago (see Fig. 8 of [101]). That increased rainfall may have increased the connectivity between the subbasins and could have resulted in a panmictic dace population in the southern Warner Valley at that time. Therefore, the results from the fast mutation rate scenario (Fig. 8) plausibly signal that the return to xeric conditions three millennia ago isolated the Western Speckled Dace in the Coleman Valley from those elsewhere in the Warner Lakes basin. Choosing definitely among these scenarios may require empirical measurement of mutation rates in *Rhinichthys*.

Under either scenario, the subdivision persisted without major change until the most recent millennium when the demographics of both lineages shifted (Fig. 8). In the Coleman subbasin, daces colonized Fosskett Spring from Coleman Creek, while in the main Warner subbasin, the Deep Creek lineage went through a strong bottleneck (Fig. 8). Because F_{ST} values between the Fosskett Spring and Coleman Creek populations broadly resemble those between the Deep and Twentymile Creek subpopulations (Table 3), we interpret the bottleneck that GADMA reconstructs in the Deep Creek lineage as representing the subdivision of Twentymile and Deep Creeks, rather than a catastrophic event that eliminated most of the individuals in the population. While we could not test that conjecture directly because GADMA cannot model more than three populations, the isolation of part of the main Warner Valley population in Twentymile Creek would certainly reduce the population remaining elsewhere in the valley.

The colonization of Fosskett Spring and the presumed split between the Twentymile and Deep Creek subpopulations falls within a period between 1,500 and 300 years ago when precipitation fluctuated around an average slightly higher than present conditions [101]. If that reconstruction is correct, then the Coleman and Crump lakebeds would have filled more regularly, allowing daces to access Fosskett Spring and creating a lacustrine connection between the Twentymile and Deep Creek stream networks. Those connections would have broken as the most recent rainy period subsided but could have re-established during particularly wet years. Thus, we interpret the low levels of contemporary gene flow that GADMA inferred between Deep Creek, Fosskett Spring and Coleman Creek as indicating intermittent and increasingly infrequent watery connections over the last 1,000 years.

However, it is critical to consider that either set of timings depend strongly upon the assumed mutation rate and generation time. Martin and Höhna [31] discussed several reasons why small populations of spring dwelling fish might experience fast mutation rates like those that Recknagel et al. [75] estimated for cichlids and which we

mirrored in our fast rate scenario. Conversely, our slow rate scenario (following [76]) corresponds closely with the most typical rates identified in a broad, recent survey of vertebrates [77]. It is also worth noting that GADMA models time in numbers of generations, not in absolute years, and thus the 588 or 896-year dates of divergence for Fosskett Dace (Fig. 8) reflect 294 or 448 generations. That generation time of two years stems from studies of the closely related *Rhinichthys osculus* in streams distant from Oregon [79, 80], and it is possible that Fosskett Dace mature or reproduce slightly sooner in their predator-free habitat. If so, the inferred dates would be closer to the present. Despite these uncertainties, these models estimate the most currently plausible dates of origin for Fosskett Dace. They colonized their isolated spring between 500 and 1000 years ago, not 10,000.

Historically, what preserved open water habitat in Fosskett Spring?

The relatively recent establishment of the Fosskett Spring population helps explain the enigma of this small population's persistence in such a tiny habitat. However, even 500 years is still more than enough time for encroaching aquatic vegetation to have rendered the spring uninhabitable. Spring-dwelling fishes often depend upon open water habitat to reproduce [103, 104], yet vegetation commonly encroaches spring systems in semi-arid environments without continued disturbance (Kodric-Brown and Brown 2007). Fosskett Dace are no exception (Fig. 4) and though they are no longer listed [38] their population has relied on regular manual removal of vegetation since the fencing of the habitat to prevent livestock access [90]. Cattle were introduced to the northern Great Basin about 150 years ago, leaving a gap of at least several centuries between the dispersal of dace to Fosskett Spring, and the arrival of European settlers and their livestock. What preserved open water habitat in Fosskett Spring during this time?

The answer likely lies in the grazing action of Bighorn Sheep (*Ovis canadensis*), which historically occupied Oregon's Great Basin [105, 106] augmented with arrival of American bison (*Bison bison*) and feral horses, which are known to reduce vegetation in Great Basin spring systems [107, 108]. Grayson [109] reported that bison appeared in the archaeological and paleontological records of south-central Oregon approximately 500 years ago during the cooler temperatures of the Little Ice Age, citing a bison tooth recovered from a dwelling within the Warner Valley dated to between 642 to 257 years ago [110, 111]. Feral horses arrived approximately 300 years ago [112]. Though the bison declined to relatively low abundance and were no longer extant by the time of European settlement [113], and the sheep were extirpated

soon after [114], the introduction of domestic livestock followed closely behind. Thus, large herbivores have been consistently present on the landscape surrounding Foscett Spring during the entire time that we estimate dace to have inhabited that water body. Indeed, the arrival of bison at or slightly after the date that we infer for colonization of the spring raise the intriguing possibility that these large ungulates were key ecosystem engineers that helped the dace to thrive in a system that vegetation had previously rendered inhospitable. Prior to modern governmental management, fires and the actions of Native Americans may also have helped to maintain open waters in Foscett Spring [107], though an ethnohistorical study conducted slightly to the north of this region concluded that human-set fires were much more prevalent in mixed conifer zones than in xeric shrub grasslands like those that surround Foscett Spring [115].

Conclusions and future directions

This study demonstrates how modern demographic analyses, rich genetic datasets and improved geographic sampling can refine and rewrite our understanding of biogeography, even in well-studied systems. By including specimens from a previously undiscovered population and by coaxing dates of divergence among populations from a large panel of SNPs, we revealed that the riddle of Foscett Dace's long persistence stemmed from an inaccurate assumption about their age and from the partitioning of knowledge about local fauna and hydrology along state lines. Though many biologists have studied Foscett Dace before (including two authors of this contribution), all had overlooked the Nevada population that provided the key to understanding the origins of one of Oregon's endemic fishes. So, our results illustrate the importance of questioning one's assumptions, and how revisiting old questions with new tools and fresh eyes can yield unexpected insights.

Foscett Dace represent just one of the many locally endemic aquatic species occurring in deserts around the world, and many of these have inspired or required intense conservation efforts. Given how much our understanding of the historical demography and biogeography of Foscett Dace shifted because of this study, it might prove informative to reevaluate our knowledge of other desert species. Are some older or younger than commonly believed? Have we missed other components of diversity in the deserts of our planet? Such studies could hold particular value in taxa for which no recent genetic studies have been performed, or where no comprehensive surveys have searched for undiscovered populations in forgotten bodies of water.

As for Foscett Dace themselves, our results reveal their genetic health and support a prediction of their

continued persistence. Genetically, they are surprisingly diverse, and appear to thrive within their spring and outflow stream. Given the existence of the refuge at Dace Spring and the regular attention that their managers place on ensuring that enough open water exists to support recruitment, their future seems secure for now. That said, monitoring should continue, and periodic estimation of heterozygosity and effective population size would help protect this isolated and potentially fragile population. Fortunately, we know now of a population of close relatives in Coleman Creek with which genes have flowed in the last several centuries. If monitoring ever reveals declining diversity in Foscett Spring, the Coleman Creek population provides an obvious reservoir of additional diversity that could be used in genetic rescue.

Finally, our results do not speak to the origins of the unusual morphology of Foscett Dace, which possess a shorter lateral line and more posterior dorsal fin than other daces in the Warner Valley. These features led Carl Bond to originally propose subspecies status for this isolated population. Hoekzema [116] confirmed the morphometric diagnosability of Foscett Dace and demonstrated that they have, on average, eight fewer pored lateral line scales than do stream-dwelling daces in the main Warner Valley. However, that study did not include Coleman Creek dace, because no one knew that they existed. Do Coleman Creek dace more closely resemble Foscett or Deep Creek dace in morphology? Does the morphometric variation result from phenotypic plasticity, or from local adaptation? If the latter, then the spring phenotype has arisen in no more than 500 generations, which is relatively rapid in evolutionary terms, though not unreasonably so [117]. If these morphologies do adapt Foscett Dace to their unusual environment, then those adaptations might also help explain their persistence [97]. The presence or absence of adaptive variation would also help reveal whether Foscett Dace have evolved substantially since their isolation. That determination could in turn inform any future debate about whether Foscett Dace qualify as a distinct population segment, an evolutionarily significant unit, or neither [118]. Answers to these questions will require morphological comparisons between Foscett Dace, Coleman Dace and dace from elsewhere in the Warner Valley, a screen of the SNP panel for loci under selection, and construction of a reference genome for *Rhinichthys klamathensis*. When complete, that work will likely reveal that this small fish in an isolated spring has not yet yielded all its secrets.

Acknowledgements

We thank Jimmy Leal, Grace Haskins and Darby Finnegan of the Bureau of Land Management for funding and advising this project. Justin Miles (ODFW) provided logistical support during the field collections. Michele Weaver (ODFW) helped to secure the Oregon collection permit, and Travis Hawks (NDOW) researched the management history of Coleman Creek at

our request. Katie Carter, Mark Dasenko, and Liz Zepeda at OSU's Center for Quantitative Life Sciences performed the lab work needed to generate raw data from the tissue subsamples. We also thank Kendra Hoekzema for sharing her thoughts about Western Speckled Dace diversity in the Great Basin, Anna Brüniche-Olsen for providing advice on demographic modeling, the DeWoody lab for providing useful feedback on earlier versions of this manuscript, and Adam Hudson for insights on the paleohydrology of the northern Great Basin. Comments from two anonymous reviewers also improved this contribution.

Authors' contributions

BLS and AB conceived the study and secured funding. BLS, HA and FM conducted fieldwork. BLS and HA prepared samples for sequencing. AB and SM analyzed the data. All authors prepared figures. BLS led the writing and editing, with SM, AB and FM contributing sections of text. All authors revised the manuscript and approved its submission.

Funding

The United States Bureau of Land Management funded this project under cooperative agreement #L20AC00103.

Availability of data and materials

Unprocessed sequence data has been deposited to NCBI's Sequence Read Archive under Bioproject PRJNA934901. All scripts and barcode files have been deposited to github (https://github.com/Andrew-N-Black/Speckled_dace). Voucher specimens have been accessioned and cataloged into the Oregon State University Ichthyology Collection (<https://ichthyology.oregonstate.edu>) where they are available to all interested researchers.

Declarations

Ethics approval and consent to participate

Fish specimens were collected from the wild in accordance with Animal Care and Use Protocol 2020–0115 approved by Oregon State University's Institutional Animal Care and Use Committee (IACUC).

Consent for publication

Not applicable to this study.

Competing interests

The authors declare no competing interests.

Received: 9 August 2023 Accepted: 17 December 2023

Published online: 04 January 2024

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