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Inferring dispersal of aquatic invertebrates from genetic variation: a comparative study of an amphipod and mayfly in Great Basin springs

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Abstract. Whether active or passive, dispersal accompanied by gene flow shapes the genetic makeup of populations and ultimately the evolutionary divergence of species. Our objective was to determine if 2 very different aquatic invertebrates with overlapping distributions show similar dispersal histories in their phylogeographic patterns and genetic uniqueness. Two spring-dwelling invertebrates, *Hyalella azteca* and *Callibaetis americanus*, were collected from 6 adjacent closed basins in the Great Basin of western North America. Cytochrome c oxidase subunit I (COI) and the 28S ribosomal subunit were used as genetic markers in *Hyalella*, and COI with the 16S ribosomal subunit of the mitochondrial genome were examined in *Callibaetis*. Maximum parsimony (MP) and likelihood (ML) analyses, F_{ST} values, analysis of molecular variance (AMOVA), Mantel tests, and nested clade phylogeographical analysis (NCPA) were used to evaluate geographical associations. *Hyalella azteca* appears to have been in the adjacent basins much longer than has *Callibaetis*. F_{ST} values in *H. azteca* reached near fixation. *Callibaetis americanus* F_{ST} values were lower suggesting greater gene flow and, consequently, higher dispersal rates. Mantel tests did not detect significant isolation by distance for either species, but NCPA on smaller networks of closely related haplotypes found the genetic structure in *C. americanus* dominated by restricted gene flow with isolation by distance. *Hyalella azteca* was characterized more by gradual range expansion followed by fragmentation. These results suggest that these isolated freshwater communities are amalgams of species that entered at different times, with weak dispersers having greater constraints on movement and, thus, reflecting an older geographical story than do species with stronger dispersal capabilities.

Key words: aquatic invertebrates, dispersal, *Hyalella azteca*, *Callibaetis americanus*, Great Basin, nested clade phylogeographical analysis.

Effective dispersal, that which results in gene flow, can shape the genetic makeup of populations and ultimately the evolutionary divergence of species. High effective dispersal among demes can result in panmixia, and thus, prevent divergence among demes. Lower levels of gene flow can favor adaptive divergence among demes or even allow the dominance of genetic drift over adaptation (Wright 1932, Templeton 2006).

Studies of dispersal often focus on frequent processes, but rare events might be much more significant (Nathan 2006). The focus on prevailing events (sensu Nathan 2006) in ecological studies results from the ease with which such events can be anecdotally observed or quantified through trapping or sampling. However, such studies often assess short distances, cannot obtain sufficient numbers needed for mark–recapture studies, cannot assess frequency of dispersal, or do not detect rare dispersal events (Bilton et al. 2001). Genetic markers provide an indirect approach that has the ability to assess effective dispersal, including those events that are rare or historical in nature (Bilton et al. 2001). Historical events might be rare, but they also can be among the most biologically significant factors structuring widely separated communities.

Dispersal should rely heavily on species-specific traits. Some taxa should be capable of greater

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dispersal among population demes than other taxa. Thus, if multiple taxa use a common habitat, the genetic diversity within their respective demes might reflect differences in their dispersal capabilities. Demes that are separated by substantial geographical barriers should have a different genetic makeup than demes that are separated by short distances and few geographical barriers.

Isolated drainage basins within the Great Basin of western North America offer a unique opportunity to compare the dispersal histories of taxa that are shared among basins. Sub-basins within the Great Basin have a long history of isolation from one another (Hubbs and Miller 1948) with faunal roots that extends at least into the early to middle Miocene (Smith 1981, Taylor 1985, Smith et al. 2002, Houston et al. 2010). An east/ west band of magmatic intrusion divides the northern and southern drainages within the Great Basin and acts as a biological barrier that is reflected in the distribution of aquatic insects (Polhemus and Polhemus 2002). The Northern Great Basin had Late Miocene to Pliocene connections with the Columbia and Snake river drainages, whereas the Southern Great Basin had more recent connections with the Colorado River (Polhemus and Polhemus 2002).

We chose to focus on aquatic systems, which, in the desert environment of the Great Basin, are of limited size and extent (Shiozawa and Rader 2005). Dispersal among aquatic systems is constrained by the lack of continuous water connections both between and within basins. However, many basins contained pluvial lakes during Pleistocene glaciations which could have provided active waterway connections in the lower elevations within basins. Two of the study basins, Steptoe Valley and Goshute Valley (Fig. 1), also were connected by a pluvial lake during pluvial maxima (Hubbs and Miller 1948, Hubbs et al. 1974). The other basins appear to have been isolated from one another at least since the early to mid Pleistocene. No natural connections occurred during the Holocene.

Despite these Pleistocene dynamics, the basins in general have undergone so much tectonic deformation and filling that it is difficult, if not impossible, to reconstruct ancient drainage paths. For example in the Bonneville Basin of Utah, the rate of valley fill is ~400 m/10^6 y (Hintze 1988). The only native fish that occurs in the western basins of our study area, *Relictus solitarius*, might have diverged from its nearest relative 4 million y ago (mya) (Houston et al. 2010), indicating considerable time for sediment deposition in these basins.

The dispersal patterns of 2 spring-dwelling invertebrates were examined. The mayfly, *Callibaetis amer-*
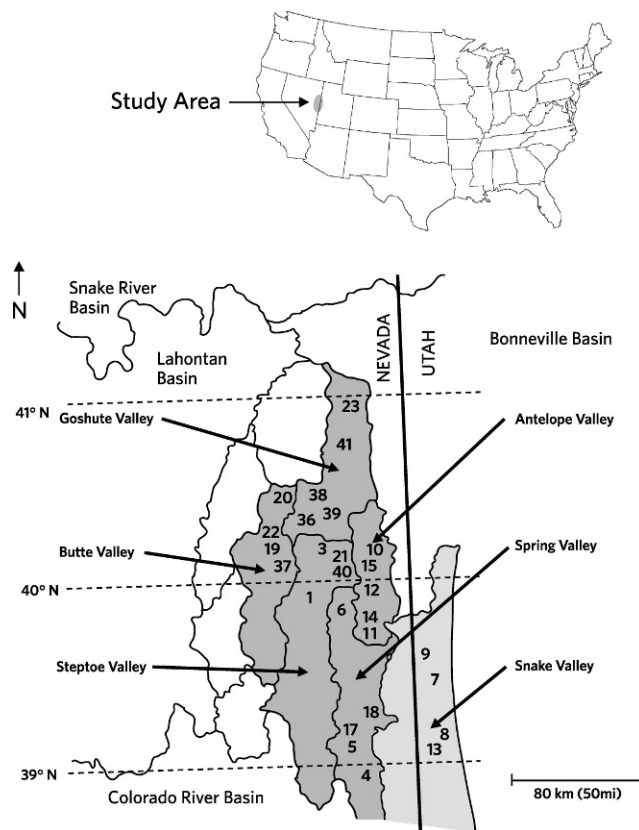

FIG. 1. Map of *Hyalella azteca* and *Callibaetis americanus* spring sites in the Great Basin listed by spring identification number (spring ID; see Table 1 for names of springs).

icanus, was chosen to represent a weak disperser, capable of overland flight, and the amphipod, *Hyalella azteca*, was chosen to represent an obligate aquatic invertebrate that is mostly limited to dispersal via direct water connections. Obligate aquatic invertebrates are more likely to be isolated for extended time periods, have low gene flow, and consequently, have more time to diverge from other populations. Aquatic insects, such as mayflies, are capable of flight, which enhances their dispersal, and thus, they are capable of colonizing springs in significantly shorter times than obligate aquatic organisms.

We predicted that amphipod populations should be genetically differentiated among springs, whereas the mayflies should be more genetically homogeneous among springs. If effective dispersal does occur, it should be more frequent within closed drainage basins than between basins. However, if dispersal occurs between basins, dispersal should be greater among basins north and south of each other rather than east to west because the geographic barriers in the Great Basin tend to be oriented north to south.

TABLE 1. Location for each population of *Hyalella azteca* and *Callibaetis americanus* including spring identification (ID) number, global positioning system (GPS) coordinates, and corresponding sample sizes for each gene. COI = cytochrome c oxidase subunit I, 28S = 28S ribosomal subunit, 16S = 16S ribosomal subunit, – indicates that no individuals were collected at that location.

Hydrographic basin	Spring ID	Spring	GPS coordinates (lat/long)	*H. azteca* COI	*H. azteca* 28S	*C. americanus* COI	*C. americanus* 16S
Snake Valley	7	Twin Springs I	39°24′12.5″N/113°51′46.5″W	–	–	37	37
	8	Knoll Springs	39°14′26.7″N/113°52′41.4″W	0	1	31	31
	9	Leland Harris Springs	39°33′11.5″N/113°53′47.6″W	29	5	–	–
	13	Caine Spring	39°08′19.4″N/114°02′55.6″W	17	15	4	3
Spring Valley	4	Spring by Spring Creek	38°57′28.0″N/114°24′32.1″W	–	–	2	2
	5	Rock Spring	39°10′51.9″N/114°22′29.9″W	–	–	6	6
	6	Spring at Rosenlund Ranch	39°50′15.5″N/114°33′42.4″W	8	13	19	19
	17	Spring east of Cleeve Creek	39°12′11.9″N/114°27′44.6″W	33	8	5	5
	18	North Millick Spring	39°18′09.4″N/114°23′19.3″W	–	–	2	2
Steptoe Valley	1	Indian Springs	39°54′17.0″N/114°40′59.4″W	1	10	–	–
	3	Flat Spring	40°04′00.2″N/114°28′51.1″W	19	9	37	38
	21	Becky Spring	40°03′49.3″N/114°35′08.1″W	–	–	8	8
	40	Cress Spring	40°03′12.3″N/114°29′29.1″W	–	–	2	2
Antelope Valley	10	Perkins Spring	40°07′20.6″N/114°24′47.3″W	–	–	3	5
	11	Blind Spring	39°50′24.9″N/114°22′16.7″W	0	4	17	17
	12	Spring by Chin Creek	40°00′55.7″N/114°22′50.0″W	65	4	–	–
	14	Tippett Spring	39°52′37.0″N/114°22′23.5″W	26	13	1	1
	15	Stockade Spring	40°03′33.1″N/114°23′38.4″W	0	7	13	12
Goshute Valley	23	Big Spring	40°59′03.7″N/114°30′16.3″W	0	6	5	8
	36	Currie Gardens	40°15′19.5″N/114°49′02.4″W	0	1	–	–
	38	Twin Springs III	40°21′08.5″N/114°49′45.3″W	0	9	7	7
	39	Mustang Spring	40°17′42.4″N/114°45′30.0″W	–	–	2	2
	41	Unnamed (Horse) spring	40°38′37.9″N/114°51′50.9″W	0	10	–	–
Butte Valley	19	Twin Springs II	40°10′10.5″N/114°59′23.0″W	9	10	–	–
	20	Quilici Spring	40°19′51.5″N/115°03′33.4″W	15	2	6	6
	22	Unnamed (S) spring	40°11′06.6″N/114°59′18.9″W	33	0	–	–
	37	Unnamed (Sound of Music)	40°01′49.4″N/114°53′54.2″W	–	–	7	8

Methods

Sampling

Our sampling was conducted in 6 adjacent closed basins in the northern-central Great Basin (Fig. 1). This sampling strategy ensured that the taxa sampled would represent lineages with ties to the Snake and Columbia river basins. During the summers of 2006 and 2007, samples were collected from 4 to 5 springs in each basin for a total of 27 sites (Table 1, Fig. 1). *Hyalella azteca* was found in 19 of those sites, and *C. americanus* in 21. Samples were collected with aquatic nets, stored in 95% ethanol, and processed and identified in the laboratory.

DNA amplification

DNA was isolated from up to 50 individuals of each population using the DNeasy protocol (Qiagen, Valencia, California). Universal primer sequences LCO and HCO for COI were used to amplify a 710-base pair (bp) fragment of the COI gene from *H. azteca* and *C. americanus* when possible (Folmer et al. 1994). Internal primers based on previously sequenced individuals of both species were used when amplification proved difficult. Primer combinations for *H. azteca* were 5587R (5587R: 5′-TAGCGCAGTCA-TTCGATCGGAGTT-3′) with HCO and 5587F (5587F: 5′-GCCCCAGCCAAATGCAAAGAAAAA-3′) with LCO. *Callibaetis americanus* primer combinations were NR (NR: 5′-AAGATTGTTAATTCGAGCTGAATT-3′) with HCO and NF (NF: 5′-ATACCAGCTAAGTG-TAATGAAAAG-3′) with LCO. Final consensus sequences for *H. azteca* were trimmed to 366 bp to include representatives from each spring, and resulting sequences have been submitted to Genbank (HM130060–HM130091). Consensus sequences for

Callibaetis were trimmed to 369 bp and resulting sequences have been submitted to Genbank (HM130092–HM130144). Amplifications were done in 40- to 50-μL total reaction volumes containing 2.0 μL of deoxyribonucleic acid (DNA) template, 5.0 μL of 10× buffer containing 15 mM MgCl₂, 5.0 μL of 1.25 mM deoxyribonucleotide triphosphates (dNTPs), 2 μL of each primer, 0.30 μL *Taq* polymerase, and the remaining volume of sterile water. The program for polymerase chain reactions (PCR) consisted of 36 cycles of 30 s at 95°C, 30 s at 48°C, 1 min at 72°C, and a 7-min extension step at 72°C.

The large ribosomal subunit, 28S rDNA, was used as a 2nd genetic marker in *H. azteca*. This nuclear gene is much more conserved than COI (Witt et al. 2006) and provided a conservative data set to compare with the phylogenetic signal from COI. Internal primers were developed to help amplify troublesome individuals. Primer sequences 3311F and 4434R (Witt et al. 2006) were paired with internal primers Rnest (Rnest: 5′-ATGCTATACTCCTTGGCCCGTGTT-3′) and Fnest (Fnest: 5′-ACCGTGAAACCGCTCAGAGTACAA-3′), respectively, to amplify a 959-bp fragment. Resulting sequences have been submitted to Genbank (HM130145–HM130157). Amplifications were done in 50-μL total reaction volumes containing 1 to 2 μL DNA template, 5 μL 10× buffer containing 15 mM MgCl₂, 5.0 μL of 1.25 mM dNTPs, 2 to 4 μL of each primer, 0.30 μL *Taq* polymerase, and the remaining volume of sterile water. The PCR program consisted of a 1-min denaturation step at 94°C, 39 cycles of 1 min at 94°C, 1 min at 51°C, 1 min at 72°C, and a 5-min extension step at 72°C.

We were unable to amplify 28S from *C. americanus*, so the 16S ribosomal subunit from the mitochondrial genome was amplified as a 2nd marker. Primer sequences S2 and 16Sar were used to obtain a 564-bp fragment of the 16S gene (Simon et al. 1994, Gieβler et al. 1999). Resulting sequences have been submitted to Genbank (HM003933–HM003975). Amplifications were done in 40- to 50-μL total reaction volumes containing 1 to 2 μL DNA template, 5 μL 10× buffer containing 15 mM MgCl₂, 5.0 μL of 1.25 mM dNTPs, 2 μL of each primer, 0.30 μL *Taq* polymerase, and the remaining volume of sterile water. The PCR program consisted of a 1.5-min denaturation step at 93°C, followed by 41 cycles of 1 min at 93°C, 1 min at 55°C, and 2 min at 72°C.

Amplified DNA was confirmed on a 1.5% agarose gel stained with ethidium bromide. DNA was cycle-sequenced using ABI Big Dye terminator protocol (Applied Biosystems, Foster City, California). The reactions were completed in 10-μL total volumes containing 2 μL of template, 1 μL of primer, 0.5 μL of Big Dye, and 6.5 μL of sterile water. Big Dye products were cleaned over Sephadex columns and dehydrated in the appropriate well of the sample plate. Sequences were obtained from the Brigham Young University (BYU) DNA Sequencing Center on either a Perkin–Elmer ABI Prism 377 automated sequencer (Perkin–Elmer, Waltham, Massachusetts) or an ABI 3100 automated sequencer. Sequences were edited and aligned using Sequencher^{TM} (version 4.8; Gene Codes Corp., Ann Arbor, Michigan). The sequences included no insertions or deletions. *Gammarus lacustris* sequences obtained from Genbank were used as outgroups for COI and 28S *H. azteca* data sets. *Baetis vernus* and *Drunella doddsi* sequences from Genbank were used as outgroups for COI and 16S *C. americanus* data sets, respectively.

Phylogenetic analyses

Haplotypes of each data set were analyzed in PAUP* v4.0b10 (Swofford 2002) using maximum parsimony (MP) and maximum likelihood (ML) analyses. MP analyses were run with the heuristic algorithm, tree bisection–reconnection (TBR) branch-swapping, and 1000 random addition sequence replicates. Strict consensuses were constructed to take into account equally parsimonious trees. One thousand bootstrap replicates were run at each node for support (Felsenstein 1985). ML analyses also were run under a heuristic search with the best-fit models selected in Modeltest 3.0 under the Akaike Information Criterion (AIC; Posada and Crandall 1998). Nodal support was obtained with 1000 bootstrap replicates.

Population genetic analyses

Pairwise sequence divergences among haplotypes were calculated in Sequencher^{TM} v4.8 and used to find intrapopulation and interpopulation divergences. Mantel and Analysis of Molecular Variance (AMOVA) tests were run in Genalex (Peakall and Smouse 2006). Mantel tests identify isolation by distance through a regression of genetic and geographic distances. Geographical distance was based on linear distance between springs because the extensive landscape evolution that has occurred in the Great Basin within the last 3 to 5 million y has erased most ancient drainage paths. AMOVA partitioned total genetic variation into % variation among basins, among spring populations, and within springs. F_{ST} values were calculated among springs using Arlequin v2.000 (Schneider et al. 2000). These values give insight into the gene flow among populations, both historical and current. As the F_{ST} value approaches 1, the alleles move toward fixation.

Values approaching 0 indicate high gene flow and mixing of alleles.

Nested clade phylogeographical analysis (NCPA; Templeton et al. 1995) was used to generate inferences on the degree of historical vs modern movement between springs. NCPA was implemented using 2 software programs, TCS v1.21 (Templeton et al. 1992, Clement et al. 2000) and GeoDis v2.5 (Posada et al. 2000). TCS uses statistical parsimony, which organizes haplotypes with low divergences into networks based on the numbers of mutational steps. We allowed up to 20 inferred mutational steps between haplotypes to increase haplotype network connectivity. Ambiguities were resolved based on topologic, frequency, and geographic criteria (Pfenninger and Posada 2002). The haplotype networks were organized into a series of nested clades (Templeton 1998). GeoDis then measured the statistical relationships of haplotypes and their spatial distribution from the distances between populations based on GPS coordinates. The statistically significant associations at $\alpha = 0.5$ were run through the inference key in GeoDis to test the null hypothesis of no association between genetic variation and geography.

Recent papers have claimed that NCPA results in too many false positives, especially in inferring isolation by distance (Knowles and Madison 2002, Garrick et al. 2008, Knowles 2008, Petit 2008). However, Templeton (2008, 2009) identified errors in their simulations and provided additional validation for NCPA. To decrease inference errors cautiously, we used multiple DNA regions to cross-validate (Templeton 2004) and included results from multiple tests (e.g., F_{ST}, AMOVA, Mantel).

Results

Hyalella azteca

Phylogenetic analyses.—We detected 43 COI haplotypes among *H. azteca*. MP analysis for COI in *Hyalella azteca* grouped most haplotypes by basin (Fig. 2). Five distinct clades were generated, 1 of which was subdivided into 2 additional clades. Two clades, Butte Valley (clade 2) and Spring Valley (clade 5), were completely confined to their respective basins. The Snake Valley clade (clade 1) contained amphipods from Leland Harris (Table 1) and Caine springs (Spring ID 13; Table 1) of Snake Valley and 1 haplotype from Indian Springs (Table 1) of Steptoe Valley.

The 3rd clade contained both Snake and Spring Valley haplotypes. The Snake Valley haplotypes, all from the Leland Harris Spring complex, were embedded within the broader Spring Valley haplotypes. The 4th clade, subdivided into the Steptoe Valley clade and
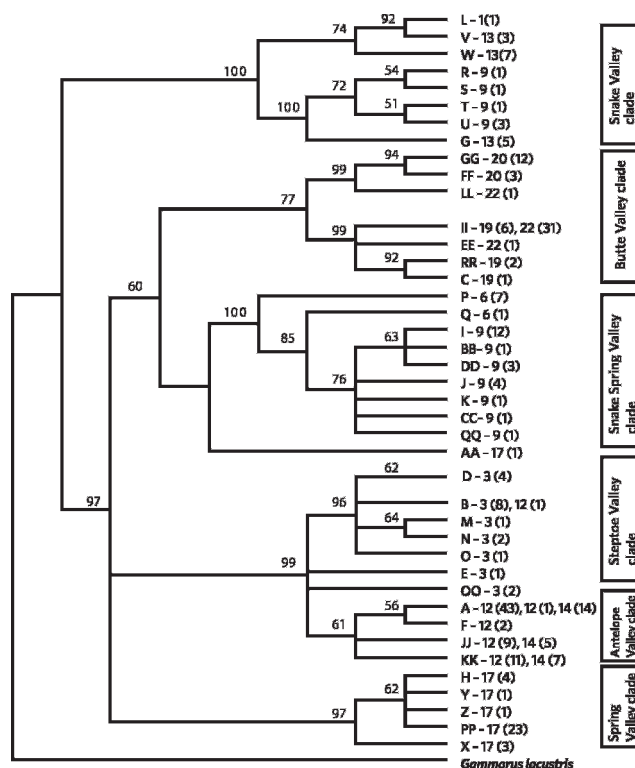

FIG. 2. Strict consensus of maximum parsimony trees of *Hyalella azteca* cytochrome c oxidase subunit I (COI) haplotypes. Numbers next to nodes are bootstrap values. Terminal branches are labeled with the haplotype identification letter code, followed by spring ID (see Table 1) with number of matching sequences in parentheses.

Antelope Valley clade (Fig. 2), was a polytomy that contained 1 weakly supported subclade (clade 4b) consisting of only Antelope Valley haplotypes.

The model of molecular evolution chosen with Modeltest for maximum likelihood analysis was unequal-frequency Kimura 3-parameter plus Γ (K81uf + Γ) for the *H. azteca* COI data set. The analysis gave results similar to MP analyses, and it indicated that Snake Valley contained the most basal lineages. In addition, Steptoe and Antelope valleys contained the most derived lineages. Snake Valley continued to have associations with Spring, Steptoe, and Antelope valleys.

Only 5 COI haplotypes occurred in multiple springs. Of those 5 cases, 3 were in springs within the same basin. The other 2 cases were a Steptoe Valley haplotype (B; Fig. 2) that occurred in Caine Spring (Table 1) of Snake Valley and an Antelope Valley haplotype (A; Fig. 2) that also occurred in Caine Spring of Snake Valley. In addition, 1 unique Steptoe Valley haplotype (L; Fig. 2) fell into the Snake Valley clade.
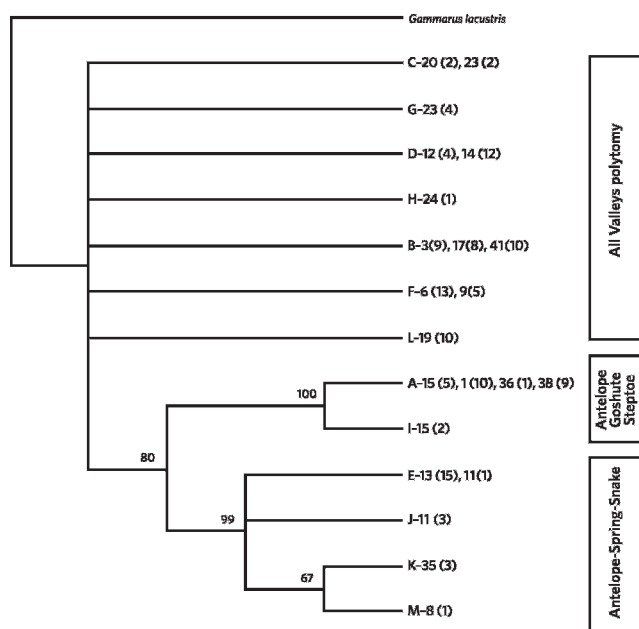
FIG. 3. Strict consensus of maximum parsimony trees of *Hyalella azteca* 28S haplotypes. Numbers next to nodes are bootstrap values. Terminal branches are labeled with the haplotype identification letter code, followed by spring ID (see Table 1) with number of matching sequences in parentheses.

Thirteen 28S haplotypes were shared among springs. MP analysis generated a polytomy (Fig. 3) with 2 well-supported subclades. One subclade contained sequences from Steptoe, Goshute, and Antelope valleys and the other contained sequences from Spring, Antelope, and Snake valleys. The model selected by Modeltest for this dataset was transversion + proportion invariant (TVM + I). The likelihood analysis strongly supported the same 2 subclades identified by the MP analysis.

Population genetic analyses.—Pairwise sequence divergences among *H. azteca* COI haplotypes ranged from 0.3 to 15.0% within springs with an average divergence of 3.7%. Sequence divergences among springs ranged from 0.3 to 22.6% with an average divergence of 12.5%. The average pairwise F_{ST} values among springs was 0.81 for COI and 0.91 for 28S. Mantel tests revealed no significant relationships between genetic distance and geographic distance among *H. azteca* COI ($R^2 = 0.0823$) or 28S haplotypes ($R^2 = 0.0462$). According to the AMOVA, 42% of the total COI genetic variation (Table 2) in *H. azteca* was explained by among-spring population variation. Thirty-nine percent was explained by among-region or basin variation, and 19% of the total variation was within spring populations. Of the total 28S genetic variation, 99% could be explained by among-spring population variation. None of the variation was attributed to among-region or basin variation, and only 1% of the total variation was within spring populations.

NCPA.—Hyalella COI haplotypes formed 6 unconnected haplotype networks in TCS. Statistically significant clades were run through the inference key. The 1st network consisted only of haplotypes from Leland Harris Spring (Table 1) in Snake Valley and had no significant inferences. The 2nd network consisted of haplotypes from Indian Springs (Table 1) in Steptoe Valley and Caine Spring (Spring ID 13; Table 1) in Snake Valley, and it also showed no association between genetic variation and geography. These 2 networks comprised the Snake Valley clade in the phylogenetic analysis (Fig. 2). The 3rd network consisted of haplotypes from Twin Springs (Spring ID 19; Table 1) and an unnamed (S) spring (Spring ID 22; Table 1) in Butte Valley. One clade in this network had an inconclusive outcome, and all other clades showed no geographical association among haplotypes. The 4th network also was in the Butte Valley clade. It contained haplotypes from Quilici Spring (Spring ID 20; Table 1) and the unnamed (S) spring in Butte Valley, and showed no association between genetic variation and geography. Collectively, the 3rd and 4th networks formed the Butte Valley clade of the parsimony analysis (Fig. 2). Clades 4-3 and 5-1 of the 5th network (Fig. 4) were characterized by restricted gene flow but with some long-distance dispersal. We could not reject the null hypothesis that clades were randomly distributed across geographic locations for all other clades in this network. Nested clade 3-1 in the 6th network (Fig. 5) was characterized by range expansion, but we could not reject the null hypothesis of no association between genetic variation and geography for the internal clades.

Hyalella 28S sequences formed 3 networks. The first 2 networks, A and B, were separated from each other by 18 mutational steps, and the 3rd network, C, was separated from networks A and B by 26 mutational steps. Network A (nested clade 3-1; Fig. 6) contained representatives from all basins sampled in our study. Network B (nested clade 2-7; Fig. 6) consisted of haplotype sequences I and A, which occurred in Indian Springs (Table 1) in Steptoe Valley, Currie and Twin springs (Spring IDs 36, 38; Table 1) in Goshute Valley, and Stockade Spring in Antelope Valley (Spring ID 15; Table 1). The inference key characterized 4 nested clades (1-1, 2-1, 3-1, 4-1) from networks A and B as having past gradual range expansion followed by fragmentation. All other nested clades showed no geographical association among haplotypes.

TABLE 2. Percentages of molecular variance (AMOVA) within springs, among springs, and among basins for each gene of *Hyalella azteca* and *Callibaetis americanus*. COI = cytochrome c oxidase subunit I, 16S = 16S ribosomal subunit.

	Hyalella azteca				*Callibaetis americanus*			
Source	COI	*p*	28S	*p*	COI	*p*	16S	*p*
Within springs	19	0.001	1	0.01	45	0.01	36	0.01
Among springs	42	0.001	99	0.01	38	0.01	31	0.01
Among basins	39	0.001	0	1.00	17	0.01	33	0.01

Network C, with haplotype sequences E, J, K, and M (Fig. 7), was analyzed separately from networks A and B. This network contained samples from Caine and Knoll springs (Spring IDs 13, 8; Table 1) in Snake Valley, Rock Spring (Spring ID 5; Table 1) in Spring Valley, and Blind Spring (Spring ID 11; Table 1) in Antelope Valley. A 1-step clade (1-1) had insufficient genetic resolution to distinguish between range expansion and restricted gene flow with some long-distance dispersal, but a 2-step and 3-step clade (2-1

FIG. 4. *Hyalella azteca* cytochrome c oxidase subunit I (COI) haplotype network A. Zero-level clades are listed as haplotype identification letter code, basin, spring ID (see Table 1), and number of matching sequences in parentheses. Nested clade 3-1 corresponds to the Spring Valley clade and nested clades 4-2 and 4-3 correspond to the Snake–Spring Valley clade of Fig. 2.
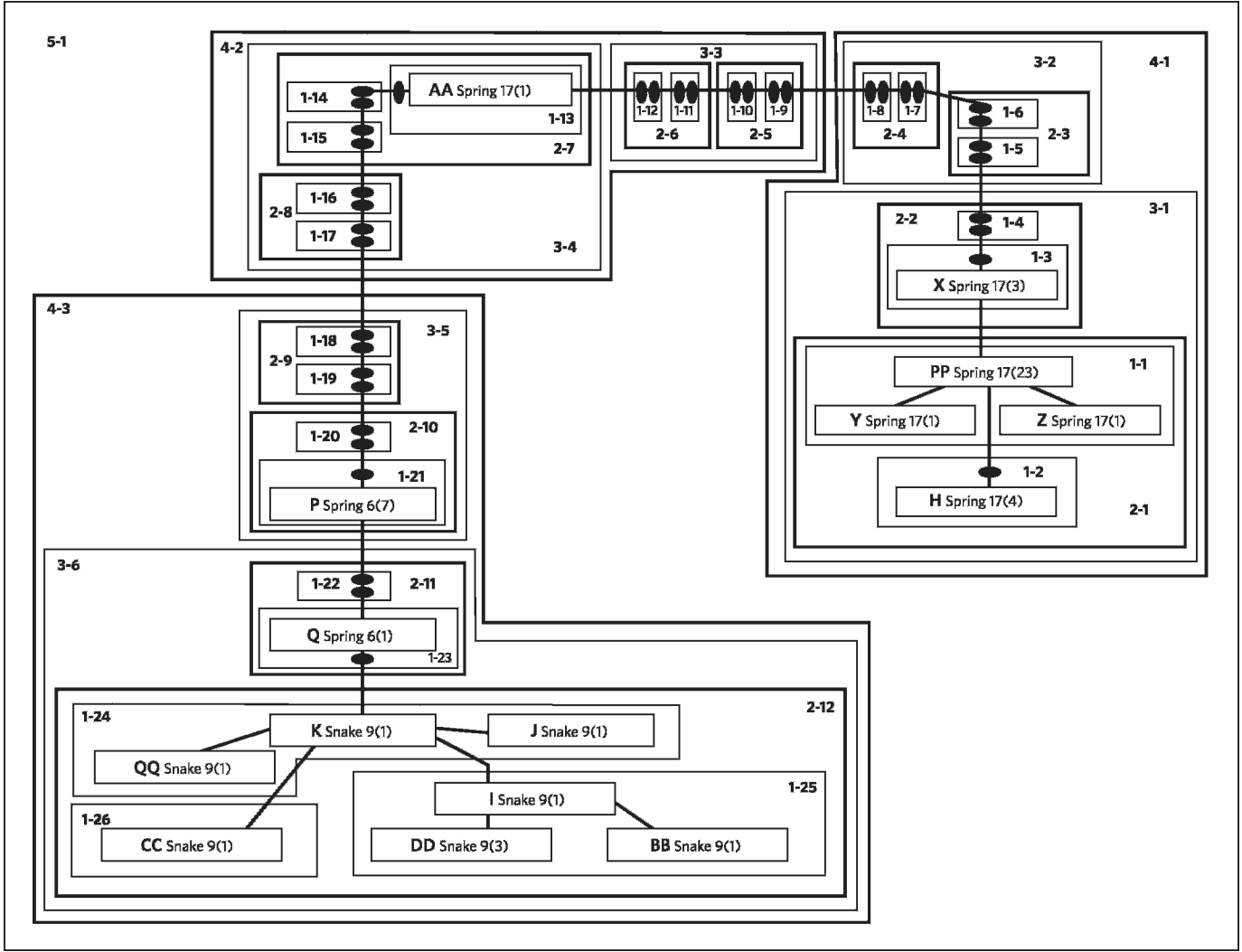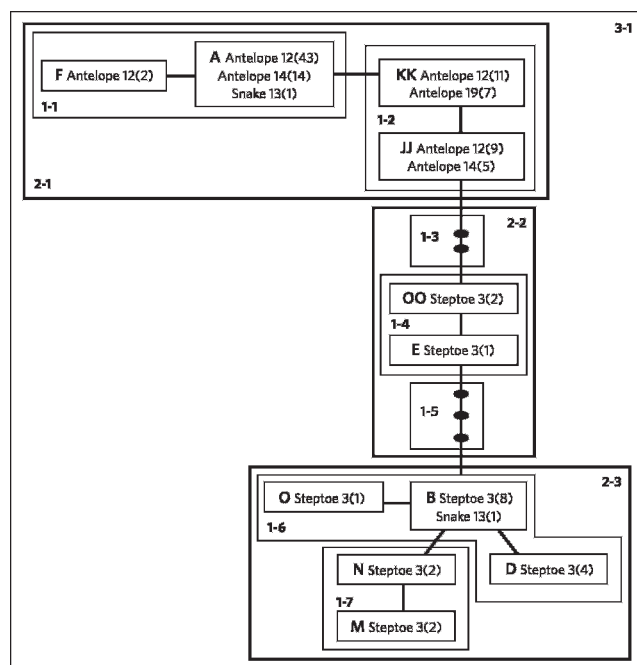
FIG. 5. *Hyalella azteca* cytochrome c oxidase subunit I (COI) haplotype network C. Zero-level clades are listed as haplotype identification letter code, basin, spring ID (see Table 1), and number of matching sequences in parentheses. Nested clade 2-1 corresponds to the Antelope Valley clade and nested clades 2-2 and 2-3 correspond to the Steptoe Valley clade of Fig. 2. Haplotype B in nested clade 1-6 occurs in both Caine Spring of Snake Valley and Flat Spring in Steptoe Valley.

and 3-1) gave evidence for past gradual range expansion followed by fragmentation.

Callibaetis americanus

Phylogenetic analyses.—The *C. americanus* 16S data set contained 43 haplotypes. MP analysis of the 16S sequences generated 5 clades (Fig. 8). The basal clade contained just 1 haplotype from Twin Spring III (Spring ID 38; Table 1) in Goshute Valley. We were unable to amplify both COI and 16S regions from all individuals, but we successfully amplified both genes from 83 individuals. These individuals allowed us to identify homologous clades in the COI and 16S phylograms. The 4 interior clades recovered from the 16S sequences matched those in the COI analysis. The only difference was that the Spring Valley clade switched position with the Antelope and Snake Valley clade, making the Antelope Valley clade the most basal.

These 2 data sets represent the same locus and generated very similar phylogenetic patterns. Therefore, we will focus our phylogenetic and nested clade

interpretations on the 16S analyses for *C. americanus*. The 16S ribosomal subunit was slightly more conserved than the COI gene and provided greater support for the deeper branches. The 16S Antelope Valley clade contained haplotypes from Antelope, Snake, Butte, Spring, and Steptoe valleys with Antelope and Snake Valley haplotypes being prominent. Interior to the Antelope Valley clade was the Spring Valley clade with only haplotypes from Spring Valley. Interior to the Spring Valley clade were the Steptoe, Spring, Antelope, and Butte Valleys clade and the All Valley clade. The best-fit model of molecular evolution selected for ML for the *C. americanus* 16S data set analysis was general time reversible + Γ + proportion invariant (GTR + Γ + I). The ML analysis was very similar to MP analysis and generated the same clades.

Population genetic analyses.—Pairwise sequence divergences among *C. americanus* COI haplotypes ranged from 0.4 to 17.1% within springs with an average divergence of 5.8%. Sequence divergences among springs ranged from 1.0 to 16.4% with an average divergence of 9.7%. The average pairwise F_{ST} values among *C. americanus* springs were 0.44 for COI and 0.52 for 16S. Mantel tests revealed no significant relationships between genetic distance and geographic distance among *C. americanus* COI or 16S haplotypes ($R^2 = 0.0947$ and 0.0234, respectively). According to the AMOVA, 45% of the total COI genetic variation in *C. americanus* could be explained by within-spring population variation (Table 2). Thirty-eight percent of the variation was explained by among-spring population variation, and 17% of the total variation was among regions or basins. Thirty-six percent of the total 16S genetic variation could be explained by within-spring population variation. Thirty-three percent of the 16S variation was explained by among-region or basin variation, and 31% of the total variation was among spring populations.

NCPA.—The 16S *C. americanus* haplotypes formed 3 separate networks (A, B, C) and 2 individual, unconnected or distantly connected, haplotypes. Network A included haplotypes in the Antelope and Snake Valley clade in the parsimony analysis (Fig. 8). Clades 1-1 and 4-1 in network A (Fig. 9) were characterized by restricted gene flow with isolation by distance. Clade 1-5 had an inconclusive outcome, and clades 2-1, 2-2, 2-3, 3-1, and 3-2 showed no association between genetic variation and geography.

Network B consisted of haplotypes from Rock Spring (Table 1) in Spring Valley. These haplotypes were part of the Spring Valley clade in the parsimony analysis (Fig. 8). A single spring contains no geographical variation, so it could not be run through the

FIG. 6. *Hyalella azteca* 28S haplotype networks A and B. Zero-level clades are listed as haplotype identification letter code, basin, spring ID (see Table 1), and number of matching sequences in parentheses. Nested clade 3-1 (network A) corresponds to the All Valleys polytomy and nested clade 2-7 (network B) correspond to the Antelope Goshute Steptoe clade of Fig. 3.

inference key. A lone haplotype, haplotype BB, also from Rock Spring in the Spring Valley clade (Fig. 8) connected to network B in 22 steps.

Network C included the Steptoe, Spring, Antelope, Butte Valleys, and the All Valleys clade (Fig. 8). This network had 2 loops that were resolved based on geography and frequency criteria. Clades 1-13 and 2-5 in network C had insufficient resolution to discriminate between range expansion and restricted gene flow (Fig. 10). Clade 2-1 gave evidence for restricted gene flow with isolation by distance. We could not reject the null hypothesis for clades 1-1, 1-2, 1-11, 1-12, and 3-2, and clade 4-1 was inconclusive. Haplotype I, from Twin Springs in Goshute Valley (Fig. 9), did not

connect to any network after 60 steps and might represent a separate species.

Discussion

Dispersal dynamics are often considered on relatively short time scales. Yet in our study, the role that landscape evolution has played in dispersal history has been important. The obligate aquatic invertebrate, *Hyalella azteca*, showed different dispersal patterns based on the gene examined. The ribosomal gene recorded its early invasion into the region, which appears to have preceded basin development. Once the basin landscape evolved, *Hyalella*, through the
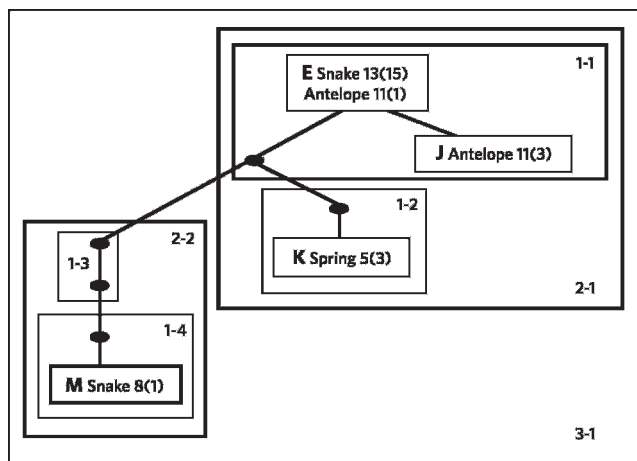
FIG. 7. *Hyalella azteca* 28S haplotype network C (nested clade 3-1). Zero-level clades are listed as haplotype identification letter code, basin, spring ID (see Table 1), and number of matching sequences in parentheses. Nested clade 3-1 corresponds to the Antelope–Spring–Snake clade of Fig. 3.

more rapidly evolving COI gene, showed characteristics of restricted gene flow and limited dispersal. In contrast, the mayfly, *Callibaetis americanus*, was a later arrival in the region, probably after basin isolation had begun. It appears to have dispersed across basin borders. Even then, its movement was strongly restricted by distance between isolated springs.

We saw little evidence to suggest that pluvial lakes were important factors in the dispersal of *Callibaetis* within basins, particularly between Goshute and Steptoe valleys, which were connected by a pluvial lake. We were unable to examine COI differences between those 2 basins in *Hyalella*, but the north and south Spring Valley *Hyalella* populations are strongly isolated despite Spring Valley also having contained an extensive pluvial lake. Two distinct COI lineages existed in Snake Valley *Hyalella* (Fig. 2). One lineage has not dispersed from its location at the northern Leland–Harris spring complex (Table 1) to springs farther to the south in Snake Valley even though the entire region was once covered by Pluvial Lake
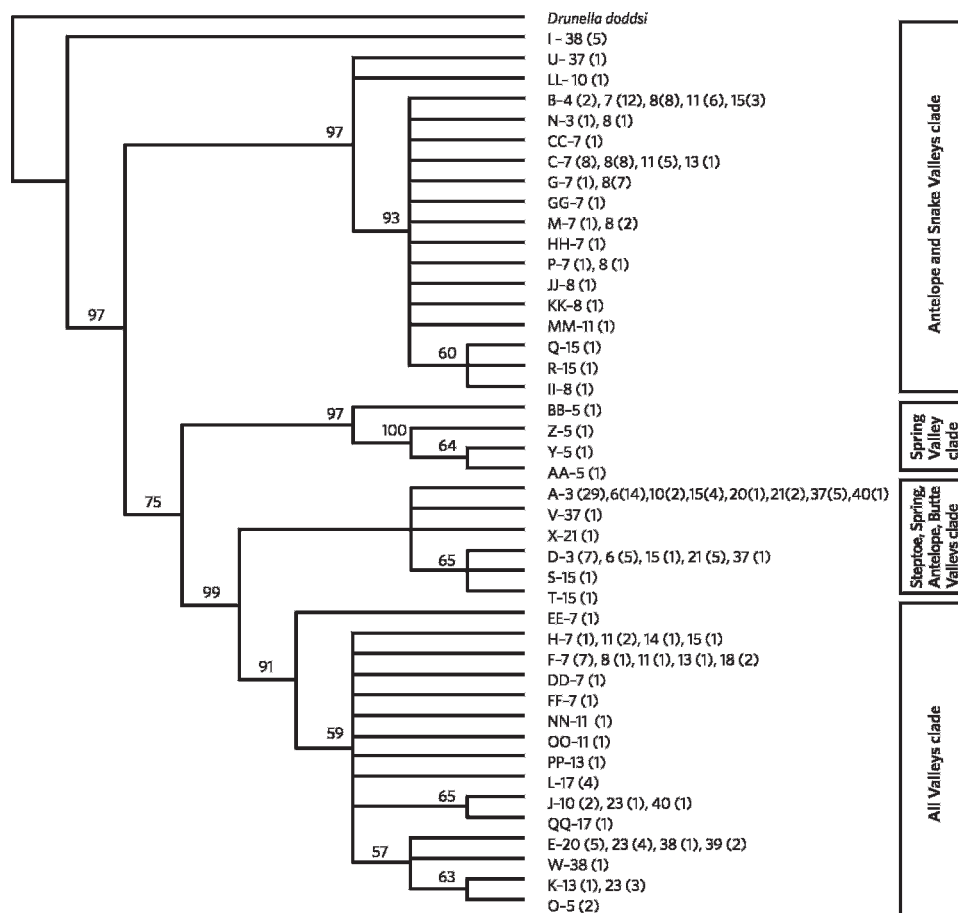

FIG. 8. Strict consensus of maximum parsimony trees of *Callibaetis americanus* 16S haplotypes. Numbers next to nodes are bootstrap values. Terminal branches are labeled with the haplotype identification letter code, followed by spring ID (see Table 1) with number of matching sequences in parentheses.
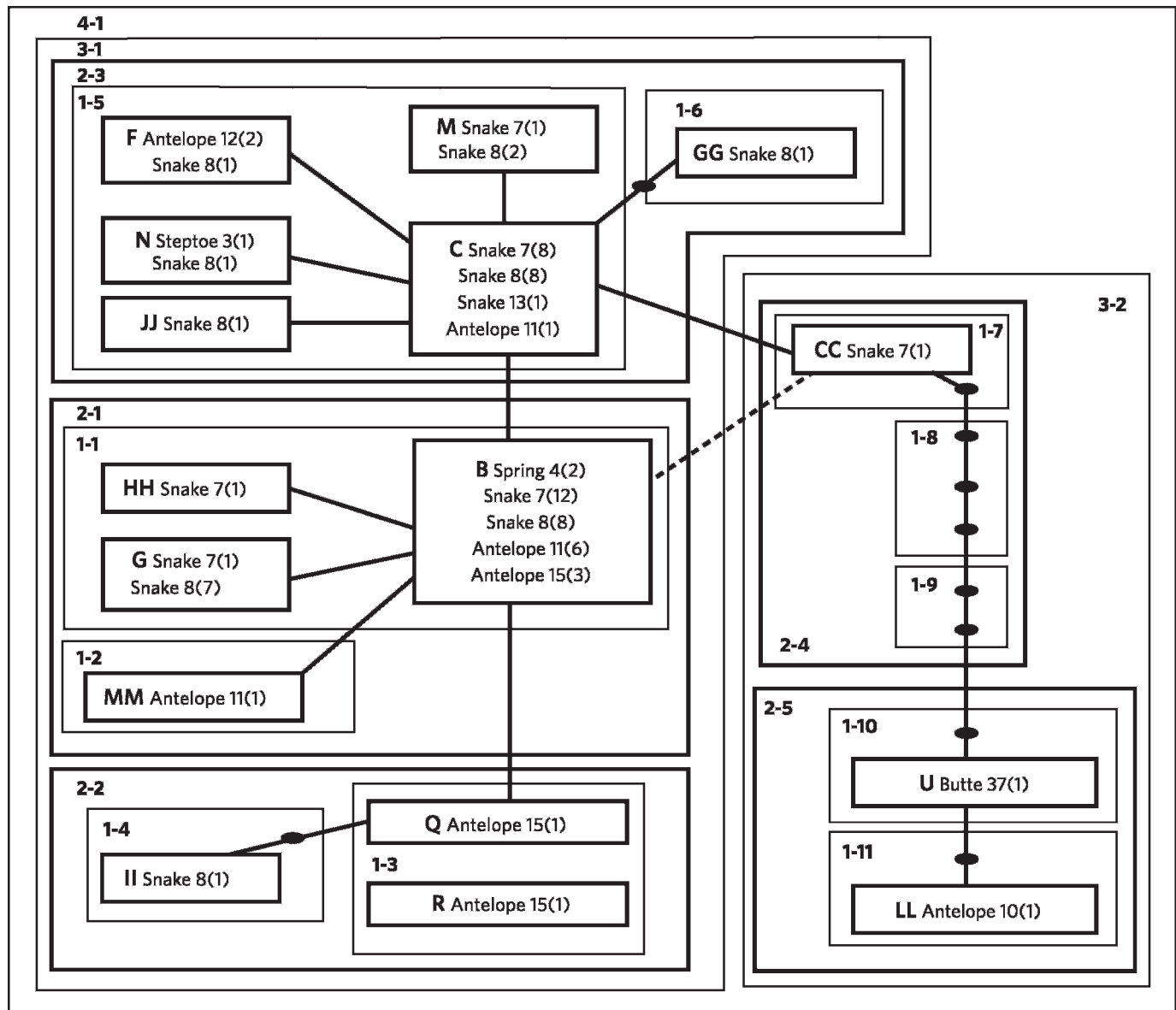
FIG. 9. *Callibaetis americanus* 16S haplotype network A. Zero-level clades are listed as haplotype identification letter code, basin, spring ID (see Table 1), and number of matching sequences in parentheses. This network corresponds to the Antelope and Snake Valleys clade of Fig. 8.

Bonneville. This result might reflect a lack of movement in pluvial lake systems. In fact, pluvial lakes might be greater barriers to the movement of aquatic organisms than one would anticipate (e.g., Taylor and Smith 1981, Taylor and Bright 1987).

Phylogenetic patterns

Parsimony and maximum likelihood analyses identified 2 major *Hyalella* lineages inhabiting the study area (Fig. 2). One consisted of haplotypes from Snake Valley and the other formed a well-supported

polytomy containing haplotypes from all 6 basins. Within the polytomy were moderately to well supported clades, which separated by basin. These relationships suggest an early separation of the ancestral *Hyalella* into eastern (Bonneville Basin: Snake Valley) and western lines (eastern Nevada basins). The only clade in multiple basins is the Snake/Spring Valley clade which indicates the subsequent dispersal event, probably from Spring Valley into Snake Valley discussed above (Fig. 2).

The Leland–Harris *Hyalella* are most closely related to the Roseland Ranch population (Spring ID 6;
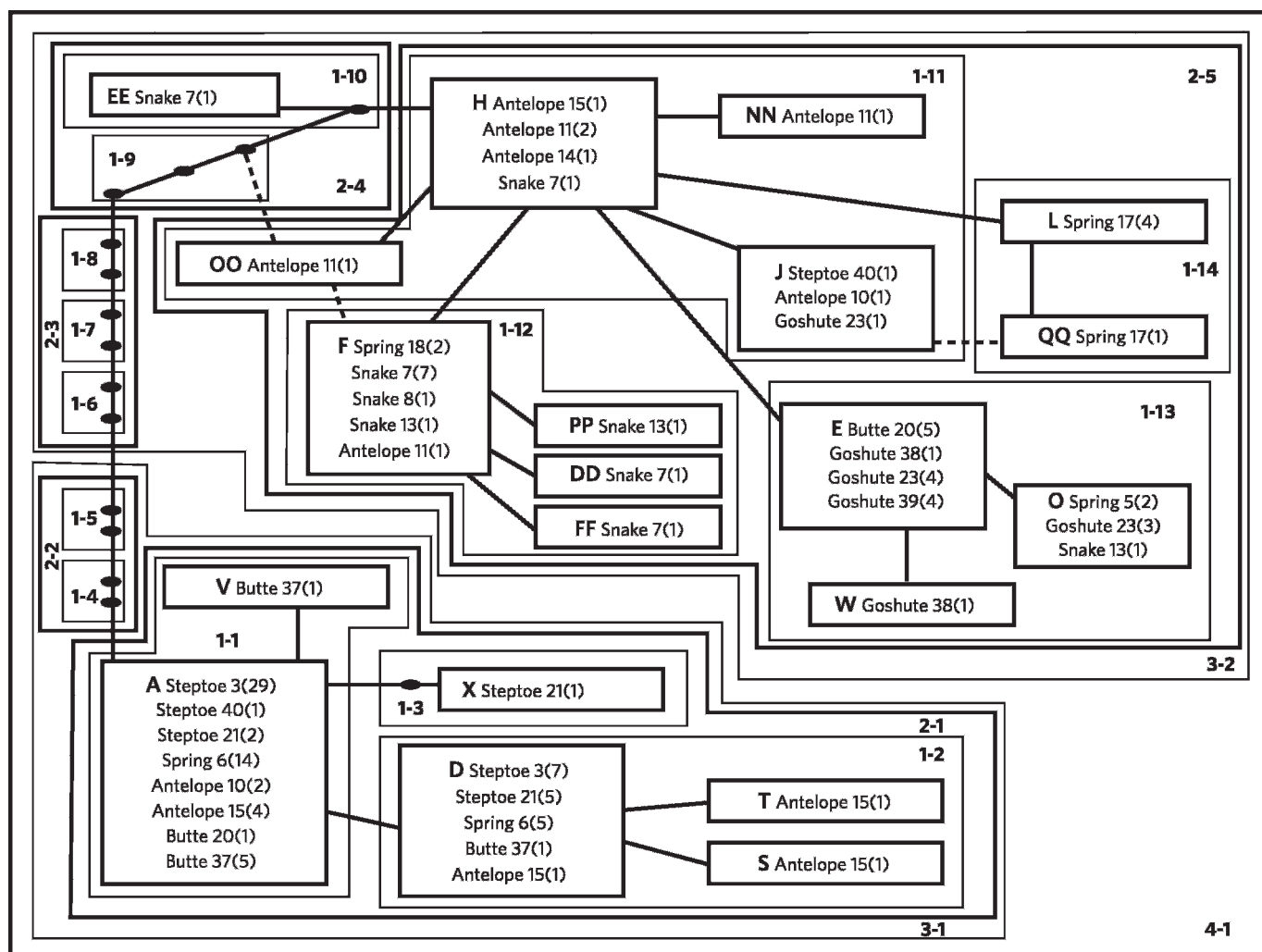
FIG. 10.   *Callibaetis americanus* 16S haplotype network B. Zero-level clades are listed as haplotype identification letter code, basin, spring ID (see Table 1), and number of matching sequences in parentheses. Nested clade 2-1 corresponds to the Steptoe, Spring, Antelope, Butte Valleys clade of Fig. 8 and Nested clade 3-2 corresponds to the All Valleys clade of Fig. 8.

Table 1) in northern Spring Valley. Based on phylogenetic associations (Fig. 2), the origin of this lineage appears to be from Spring Valley. Hubbs and Miller (1948) noted the lack of evidence that Pluvial Spring Lake was connected to the Bonneville Basin during the latest pluvial maximum, and concluded that any interbasin connections with Spring Valley were probably at the southern end of the valley. Our data suggest that a transfer did occur at the northern end of the valley, but we have no way of dating the event at this time. Hubbs and Miller (1948) reported collecting a sucker (apparently a mountain sucker) in Spring Valley (Smith 1966). If this specimen was native, its presence might reflect a transfer from the Bonneville Basin to the Spring Valley Basin. Future studies might clarify these contrasting data.

Mitochondrial-based phylogenetic analyses of *H. azteca* provided evidence of divergence within basins, but the nuclear gene did not. Nuclear genes, especially the transcribed ribosomal subunits, evolve much more slowly than mitochondrial genes (Brown et al. 1979, 1982) and reflect much older events. The 28S phylogeny consists of a major polytomy among lineages with 2 well supported subclades on 1 of the branches (Fig. 3). These subclades do not separate by basins, indicating that lineage extinction has not occurred with this gene, although some regional differentiation has taken place. Collectively, the 2 *Hyalella* data sets show 2 different points in the taxon's evolutionary history. COI suggests more recent vicariance and 28S records dispersal dynamics before the populations became isolated by basin development.

Insight into the timing of these dispersal events can be gained from the Relict dace, *Relictus solitarius* (Pisces:Cyprinidae). This species, the only fish native to Steptoe, Butte, and Goshute valleys, split from the Colorado pikeminnow/Utah chub-roundtail chub (*Gila atraria-Gila robusta*) lineage in the early to mid-Miocene, ~9.2 mya, and from *Gila orcutti* 5 to 6 million years later (Houston et al. 2010). If the amphipods entered the western basins with *Relictus*, they, and the fish, would have been isolated subsequently in separate basins as the landscape evolved under them.

Phylogenetic analyses of both COI and the 16S genes from *C. americanus* generated 4 major clades (Fig. 9). The evidence for dispersal among combinations of basins indicates that the mountain ranges in the Great Basin are not strong geographic barriers to *Callibaetis* dispersal. This failure of the mountain ranges to be strong barriers could be related to the ability of the mayfly to cross over mountains, or dispersal across the north–south borders of these basins might be followed by rapid, within-basin colonization so that no clear dispersal signal remains.

Genetic diversity

Our COI based mitochondrial sequence divergences for *H. azteca* is high, but consistent with published values. Witt et al. (2006) reported 4.4 to 29.9% sequence divergence among provisional species. We did not try to delineate provisional species, but we documented 0.3 to 22.6% sequence divergence between our spring populations. Clearly cryptic species exist in these basins.

Sequence divergence estimates have not been reported for *C. americanus*, but COI sequence divergences in leptophlebiid mayflies have been shown to be as high as 9.9 to 18.7% among phylogenetic lineages from ≥ 1 river catchments (Baker et al. 2004). The average within-spring mitochondrial COI sequence divergences (5.8%) for *C. americanus* in our study were higher than the within-spring sequence divergence of *H. azteca* (3.7%), but the 9.8% between-springs COI sequence divergence in *C. americanus* was considerably lower than that of *H. azteca*. These data suggest that mayfly flight results in greater gene flow, and thus, higher within-spring genetic divergence. *Hyalella*'s dispersal requirements restrict its movement, favoring less within-spring divergence. The spring habitat probably facilitates *H. azteca* lineage extinction within populations as well.

Hyalella azteca's mean F_{ST} value was very high, and many pairwise comparisons were near fixation and indicative of essentially no gene flow. *Callibaetis americanus* had a mean F_{ST} value ~½ of that of *H. azteca*, but still indicative of very great genetic differentiation (Hartl and Clark 1997). This result mirrors the sequence divergence data, but it also suggests that, despite its mobility, the mayfly still shows significant restriction in gene flow. *Callibaetis americanus* is a weak disperser, and the weak flight capabilities of mayflies in general might be a significant barrier to effective dispersal in desert settings.

AMOVA showed that *H. azteca* has high among-population genetic variation, consistent with isolated populations that have limited dispersal ability (Kelly et al. 2002), whereas *C. americanus* has greater within-population genetic variation, consistent with higher dispersal rates. Other studies on aquatic insects have shown similar higher within population genetic variation (Bunn and Hughes 1997, Miller et al. 2002).

Dispersal inferences

Witt et al.'s (2008) COI-based phylogeny of *H. azteca* suggested that dispersal events explained amphipod relationships. Most of the inferences in our NCPA for the *Hyalella* COI data set indicated insufficient data or inconclusive results, primarily because of too few samples or limited geographic distribution of haplotypes within the tested nested clades. The limited geographic distribution, in turn, is the result of the high genetic divergence among lineages, which did not allow many of the haplotypes to be included together in a single NCPA even though a relatively large number of individuals was sampled. Of the 2 nested clades that included a range of haplotypes from multiple basins (Figs 4, 5), the higher-step clades detected restricted gene flow with some long distance dispersal (Spring–Snake Valleys) and range expansion (Antelope–Steptoe Valleys). In contrast, inferences from *H. azteca* 28S networks showed, especially in the higher-step clades, gradual range expansion followed by fragmentation. This result supports the observations made with the phylogenetic analyses and the results of the AMOVA and *F* statistics.

The haplotype networks generated by the *C. americanus* 16S data set corresponded to the 3 major clades generated by MP analysis (Fig. 8). The Mantel tests did not detect significant isolation by distance when evaluating all haplotypes together, but NCPA examined smaller networks of related haplotypes and was able to detect isolation by distance.

Evidence of recent transfers

Three anomalous *Hyalella* haplotypes were detected. The Snake Valley eastern clade contained 1 haplotype differing by just 2 mutational steps from

its Caine Spring (Spring ID 13; Table 1) haplotypes. The haplotype was collected in Indian Springs (Table 1) of Steptoe Valley. The fact that the haplotypes were not identical leaves the origin of the Indian Springs haplotype open to interpretation. The origin could have been a recent natural invasion from Snake Valley, or the haplotype could have been introduced by man through a water transfer from Snake Valley. It is unclear how the haplotype could have crossed Spring or Antelope Valley without leaving additional genetic information behind, but if the former is the case, then lineage extinction might have occurred in the intervening valley(s), or sampling was insufficient to detect the haplotypes. If the latter is the case, then additional sampling probably would locate the same haplotype in Caine Spring. Caine Spring also contained haplotypes matching those in clades from 2 other basins, Flat Springs (Spring ID 3; Table 1) in Steptoe Valley and Tippett Spring (Spring ID 14; Table 1) and an unnamed spring by Chin Creek (Spring ID 12; Table 1) in Antelope Valley. The 2 haplotypes could have invaded Caine Spring naturally, but we think they represent recent transfers by man because they show no mutations from the lineages of origin, and no clades of similar haplotypes were collected in Caine Spring.

Applications

Beyond generating insights into the roles of dispersal and history in the structuring of aquatic communities, these types of studies can have a strongly applied side. A major threat to the survival of aquatic species is habitat loss, destruction, and fragmentation (Dudgeon et al. 2006). In today's Great Basin, species are facing this threat in the form of groundwater development. Dispersal–recolonization studies typically have focused on stream-dwelling invertebrates (Tronstad et al. 2007, Chaput-Bardy et al. 2008, James et al. 2008). However, spring invertebrates in desert basins occur in discrete sites often distantly isolated from other water sources. They cannot rely on a recolonization cycle for persistence (i.e., Müller 1954) or colonization from nearby streams. Instead geological history has played a significant role in their modern distribution, and their presence or colonization of springs requires time periods of hundreds of thousands to millions of years. Those taxa that cannot disperse rapidly, especially obligate aquatic organisms, but also, as seen with the mayfly, some insects will be pushed into extinction if their habitat is dried for even a short time. With that, we will lose the rich evolutionary history that the organisms have recorded in their genetic code.

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