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Dispersal ability and genetic structure in aquatic invertebrates: a comparative study in southern California streams and reservoirs

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SUMMARY

1. The natural seasonal drying and flooding of southern California streams have been altered over the past century by activities related to agriculture, flood control, and reservoir construction. The genetic structure and diversity of aquatic invertebrates inhabiting these environments is largely unexplored, and may be important for conservation.
2. We sampled two species of aquatic invertebrates with different dispersal abilities to assess genetic structure and diversity, and make inferences about the evolutionary processes that underlie these genetic patterns. The mayfly *Fallceon quilleri*, which has a winged terrestrial stage, was sampled from perennial and intermittent streams from three catchments across San Diego County. The amphipod *Hyalella azteca* was sampled from streams (perennial and intermittent) and reservoirs in a single catchment (San Dieguito). Because it is obligately aquatic throughout its life-cycle, *H. azteca* was assumed to disperse less than *F. quilleri*.
3. Intrapopulation and overall genetic diversity was higher in *F. quilleri* than in *H. azteca*. In *F. quilleri* there was very little genetic divergence among populations, and most of the genetic differentiation that was observed could be attributed to a single population. In *H. azteca*, populations were markedly differentiated between the upper and lower segments of the San Dieguito basin, which are separated by a *c.* 10 km section of stream that rarely has surface flow. Within both segments, genetic divergence between sites connected by reservoirs and perennial streams was not significantly different.
4. Our results suggest that *F. quilleri* disperses widely and thus avoids genetic bottlenecks and marked levels of population differentiation that may be expected from frequent extinctions and recolonizations. In contrast, restricted dispersal in *H. azteca* is associated with relatively low genetic diversity and high genetic divergence across a portion of the catchment in which surface flow is rare.

Keywords: amphipod, biodiversity, freshwater invertebrates, mayfly, population genetics

Introduction

Molecular markers are commonly used to assess levels of population genetic structure and diversity, and make inferences about gene flow in aquatic invertebrates. It is generally assumed that genetic divergence will be greater in populations that are

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completely isolated, or have restricted gene flow (Slatkin, 1985). Although the accuracy of estimates of dispersal and gene flow derived from population genetic statistics is debatable (Bossart & Prowell, 1998), some population genetic statistics correlate predictably with dispersal ability across a wide variety of taxa (reviewed by Peterson & Denno, 1998; and by Bohonak, 1999). In invertebrates, empirical studies have shown that species with high dispersal ability are, in general, more genetically homogeneous across their range than those with limited dispersal ability (e.g. Jones *et al.*, 1981; Miller, Blinn & Keim, 2002; Gervasio *et al.*, 2004).

Invertebrates inhabiting streams in arid regions face conditions that regularly subject populations to local extinction. In arid southern California, many streams seasonally undergo extreme changes in flow, ranging from completely dry during the summer to flooding during the wet winter (Gasith & Resh, 1999). These extreme flow fluctuations can have adverse affects on aquatic invertebrate populations. During the dry season, populations inhabiting intermittent streams either go extinct, move to other streams, or have a resistant stage such as a diapausing cyst, while dramatic reductions in population size often result from flooding events (Meffe & Minckley, 1987; Gasith & Resh, 1999).

Although it is clear that natural extinction and recolonization cycles in many streams have been altered by human induced changes to the natural flow regime, the effects of these changes on ecological and evolutionary population dynamics is largely unexplored. For example, many streams that were once intermittent are now perennial, because of agricultural and urban runoff. Reservoirs created for water storage have altered the natural hydrological cycles, and changed stream connectivity patterns. These changes may be altering natural patterns of genetic structure, either increasing or decreasing gene flow compared with historic levels (Meffe & Vrijenhoek, 1988). In San Diego County, these changes are further complicated by water imported from the Colorado River and northern California, potentially transferring aquatic organisms and allowing for gene flow between distant, historically isolated catchments.

Together with gene flow and drift, the metapopulation dynamics of periodic extinctions and recolonizations define genetic diversity and structure in systems where these dynamics occur (e.g. Slatkin, 1985, 1987). Wade & McCauley (1988) demonstrated that the effects of extinction and recolonization on population structure depend on the number of colonists and their origins. If new populations are founded by many individuals from numerous populations, then population turnover will have a homogenizing effect on population differentiation. If populations are founded by a few colonists from a limited number of source populations; however, metapopulation dynamics can dramatically increase genetic differentiation. Furthermore, extinction and recolonization affect intrapopulation genetic diversity. After an extinction or severe reduction in population size, genetic diversity in a population can decrease through a bottleneck effect. (Nei, Maruyama & Charkraborty, 1975; Gilpin, 1991; Harrison & Hastings, 1996; Pannell & Charlesworth, 2000; Wakeley, 2000).

Here, we used mitochondrial DNA (mtDNA) sequence data to study genetic structure and diversity in two aquatic invertebrates that are common and abundant in coastal southern California streams and reservoirs. We sampled the mayfly *Fallceon quilleri* (Dodds) (Ephemeroptera, Baetidae) and the amphipod *Hyalella azteca* (Sassure) (Crustacea, Amphipoda) because their differing life-histories and dispersal abilities make them ideal species for a comparative study.

Despite considerable work on the population biology of mayflies, little is known specifically about *F. quilleri* and this study is the first to analyse its population genetic structure. Like most other aquatic insects, mayflies spend most of their lives as larvae in the stream and emerge in a short-lived (a few hours to a few days) winged adult stage to reproduce (Merritt & Cummins, 1996). It is widely accepted that larval movement within the stream and adult flight across the terrestrial landscape are the primary mechanisms for dispersal in aquatic insects. Studies have suggested that adult dispersal in mayflies is widespread (e.g. Gibbs *et al.*, 1998; Smith & Collier, 2001). However, studies of mayflies and other stream insects in Australia have shown low genetic differentiation over large spatial scales, despite high levels of genetic differentiation within streams. Schmidt, Hughes & Bunn (1995); Bunn & Hughes (1997) and Hughes *et al.* (2000) suggest that dispersal via adult flight largely shapes genetic structure on the largest scale, while small-scale

differentiation is driven by a limited number of matings at each site.

In contrast to mayflies, freshwater crustaceans have no winged terrestrial stage and dispersal is presumably limited to within stream movements. Some taxa have adapted to survive in poor environmental conditions normally through diapausing or cyst stages. However, *H. azteca* does not possess any such adaptation (Thorp & Covich, 1991). Molecular studies have demonstrated that stream macrocrustaceans frequently show high levels of genetic differentiation over small geographic areas (Hughes *et al.*, 1996; Hurwood & Hughes, 2001). In *H. azteca*, levels of genetic divergence are so high that *H. azteca* is hypothesized to be a cryptic complex of several morphologically similar species (Hogg *et al.*, 1998; McPeek & Wellborn, 1998; Witt & Hebert, 2000; Gonzalez & Watling, 2002; Witt, Threloff & Hebert, 2006).

For this study, we hypothesized that active dispersal in *F. quilleri* leads to the avoidance of bottlenecks and population subdivision, even among streams that are characterized by seasonal drying and flooding and consequent local extinctions. We specifically predicted that genetic differentiation in this species would be detected only at the largest spatial scale, and that genetic diversity would be similar in intermittent and perennial streams. In contrast, seasonal extinctions of *H. azteca* populations in intermittent streams should lead to more severe genetic bottlenecks, because recolonization probably involves fewer individuals (from permanent populations) over a more restricted area. We also predicted high levels of genetic differentiation in *H. azteca* among sites not connected by permanent water sources.

Methods

Study organisms

Fallceon quilleri was collected between January 2004 and June 2005 from 18 intermittent and perennial streams in three catchments in San Diego County, CA (Tijuana, San Dieguito and Santa Margarita: Fig. 1, Table 1). Distances among the three catchments are comparable with those over which genetic differentiation has been detected in previous studies on mayflies (Gibbs *et al.*, 1998; Smith & Collier, 2001; Hughes *et al.*, 2003). All accessible streams in the three catchments were surveyed and *F. quilleri* was collected from a stream if it was present. Most streams within each catchment were surveyed more than once during this time, often in different seasons. *Fallceon quilleri* was generally present during periods of high flow, and absent during low flow or when the stream had been reduced to static pools.

We initially sampled *H. azteca* from the same catchments from which *F. quilleri* was collected. We were forced to focus our sampling efforts on the San

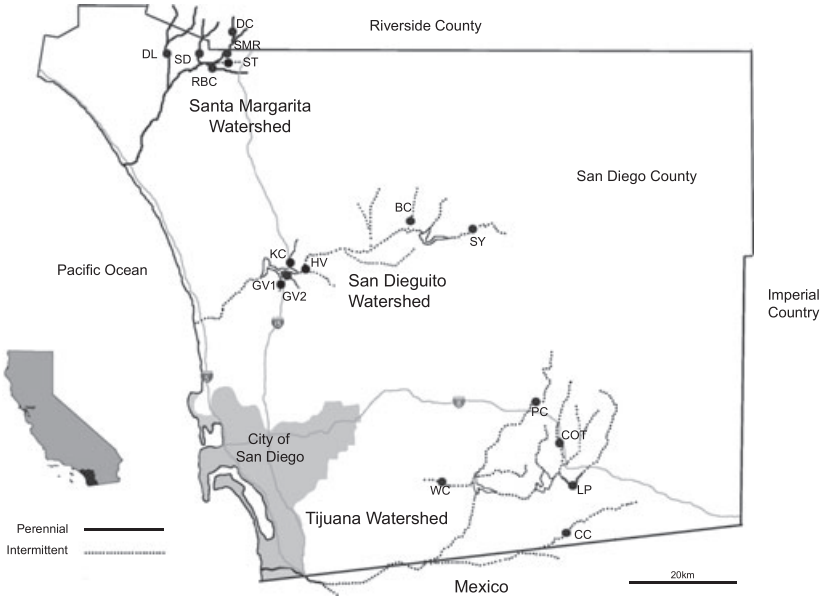

Fig. 1 Map showing sampling sites for *Fallceon quilleri* in San Diego County (inset is San Diego County, California). Abbreviations (ST, GV2, etc.) are sampling sites (see Table 1).

Table 1 Sampling locations and genetic diversity indices for *Fallceon quilleri*

Site	Site abbreviation	Catchment	Permanence	n	θ_K	H_e
Pine Valley Cr.	PC	Tijuana	Intermittent	6	1.700	0.600
Cottonwood Cr.	COT	Tijuana	Intermittent	2		1.000
La Posta Cr.	LP	Tijuana	Perennial	7	6.400	0.905
Wilson Cr.	WC	Tijuana	Intermittent	6	11.442	0.933
Campo Cr.	CC	Tijuana	Intermittent	6	1.700	0.733
Santa Ysabel Cr.	SY	San Dieguito	Intermittent	4	3.766	0.833
Boden Canyon Cr.	BC	San Dieguito	Intermittent	6	4.063	0.867
Highland Valley Cr.	HV	San Dieguito	Intermittent	6	1.670	0.800
Kit Carson Cr.	KC	San Dieguito	Perennial	5		1.000
Hodges Reservoir trib.	GV1	San Dieguito	Perennial	5	2.225	0.700
Hodges Reservoir trib.	GV2	San Dieguito	Perennial	5	7.106	0.900
San Dieguito R., upper	SDR1	San Dieguito	Intermittent	7	3.029	0.810
Devils Canyon Cr.	SMRDC	Santa Margarita	Perennial	6	11.442	0.933
Santa Margarita R.	SMR	Santa Margarita	Perennial	7	1.423	0.524
Stone Cr.	ST	Santa Margarita	Intermittent	6	11.442	0.933
Rainbow Cr.	RBC	Santa Margarita	Perennial	6	4.063	0.867
Sandia Cr.	SD	Santa Margarita	Perennial	5	7.106	0.900
DeLuz Cr.	DL	Santa Margarita	Perennial	8	2.501	0.821

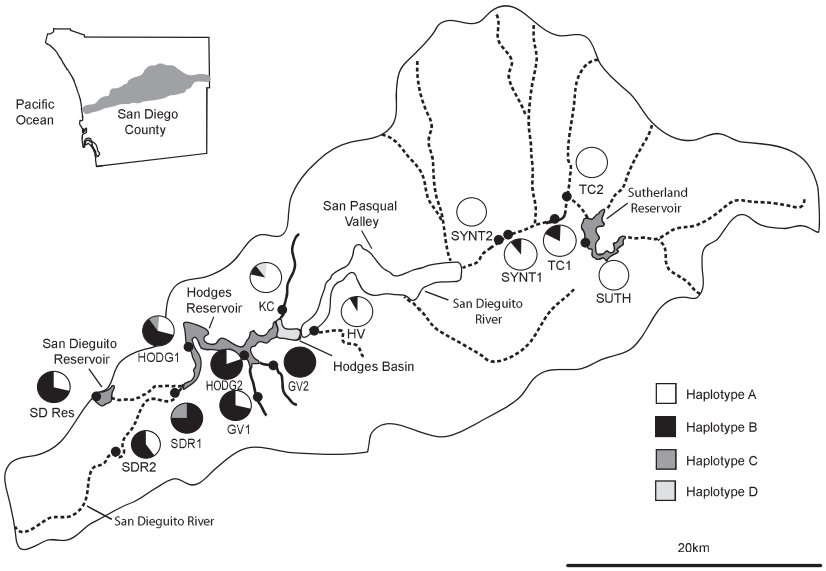

Fig. 2 Map showing sampling sites for *Hyalella azteca* in the San Dieguito catchment. Pie charts represent haplotype frequencies at each site (inset is San Dieguito catchment).

Dieguito catchment only, however, after exhaustive attempts to sequence individuals from the Santa Margarita and Tijuana catchments failed (i.e. modifying original primers, developing entirely new primers and modifying extraction techniques). This obstacle might have been due to a major genomic change, and could indicate the presence of a cryptic species, as has been documented in other studies on *H. azteca* (Hogg *et al*., 1998; McPeek & Wellborn, 1998; Witt & Hebert, 2000). The San Dieguito catchment contains three reservoirs connected by perennial and intermittent streams. From this catchment, we collected individuals from 14 locations: 10 streams (six intermittent, four perennial) and three reservoir sites (Fig. 2, Table 2). Because of its large size, two locations were sampled in Hodges Reservoir.

Invertebrates were sampled using a D-frame kick net. *Fallceon quilleri* and *H. azteca* were sorted from the sediment and transported back to the laboratory alive in cold stream water. Species were identified under a dissecting microscope using keys from Merritt & Cummins (1996) for *F. quilleri* and Thorp

Table 2 Sampling locations and diversity indices for *Hyalella azteca*

Site	Site abbreviation	Catchment	Catchment segment	Permanence	n	θ_K	H_e
Sutherland Reservoir	SUTH	San Dieguito	Upper	Reservoir	8	0.000	0.000
Temescal Cr., upper	TC1	San Dieguito	Upper	Intermittent	7	0.000	0.000
Temescal Cr., lower	TC2	San Dieguito	Upper	Perennial	6	0.592	0.333
Santa Ysabel Cr. at north trails, upper	SYNT1	San Dieguito	Upper	Intermittent	10	0.430	0.200
Santa Ysabel Cr. at north trails, lower	SYNT2	San Dieguito	Upper	Intermittent	8	0.000	0.000
Highland Valley Cr.	HV	San Dieguito	Upper	Intermittent	13	0.379	0.154
Kit Carson Cr.	KC	San Dieguito	Upper	Perennial	9	1.137	0.417
Hodges Reservoir trib.	GV1	San Dieguito	Lower	Perennial	10	0.430	0.467
Hodges Reservoir trib.	GV2	San Dieguito	Lower	Perennial	9	0.000	0.000
Hodges Reservoir, upper	HODG1	San Dieguito	Lower	Reservoir	9	0.455	0.389
Hodges Reservoir, lower	HODG2	San Dieguito	Lower	Reservoir	4	0.879	0.500
San Dieguito Reservoir	SDRES	San Dieguito	Lower	Reservoir	7	0.530	0.476
San Dieguito R., upper	SDR1	San Dieguito	Lower	Intermittent	10	1.052	0.600
San Dieguito R., lower	SDR2	San Dieguito	Lower	Intermittent	10	0.430	0.533

& Covich (1991) for *H. azteca*. Individuals were placed in 1.5 mL Eppendorf tubes and frozen at −80 °C until genetic analyses.

DNA analysis

Fallceon quilleri DNA was extracted from each individual using Qiagen DNeasy (Qiagen, Valenica CA, U.S.A.) or BioRad Aquapure (Bio-Rad Laboratories, Hercules, CA, U.S.A.) genomic DNA isolation kits. We amplified a 657 base pair region from the mitochondrial gene cytochrome oxidase subunit 1 (CO1). The primers used for amplification were HCO 2198 from Folmer *et al.* (1994) (5′-TAAACTTCAGGGTGACCAAAAAATAC-3′) and a primer developed in the Bohonak laboratory similar to LCO 1490 (Folmer *et al.*, 1994) named ''Jyothi'' (5′- TTCTCAACAAATCATAAAGATATTGG -3′). Polymerase chain reaction (PCR) reactions contained 0.6 μL of 25 mM $MgCl_2$, 2.0 μL 8 μM dNTPs, 2.0 μL of 10x PCR buffer, 0.85 μL of each primer (10 μM), 0.078 μL *Taq* and 2 μL of DNA template. The reaction was adjusted to a total volume of 20 μL by adding sterilized water. PCR was performed with a TGradient thermocycler (Biometra, Goettingen, Germany). DNA was initially denaturated at 94 °C for 2 min, followed by eight cycles at 94 °C for 30 s, 47 °C for 40 s stepped up to 49.8 °C and a 1-min extension at 72 °C. This was followed by 32 cycles at 94 °C for 30 s, 50 °C for 30 s and a 1-min extension at 72 °C. The cycle ended with a final elongation at 72 °C for seven minutes. PCR products were visualized electrophoretically on a 2% agarose gel using ethidium bromide staining. PCR products were purified using the Geneclean Turbo purification kit (Qbiogene, Irvine, CA, U.S.A.), cycle sequenced with primer 'Jyothi' using the BIGDYE v. 3.1 termination mix, and cleaned with G-50 Sephadex following manufacturer's instructions (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, U.S.A.). DNA was sequenced using an ABI Prism 377 automated sequencer (Applied Biosystems Foster City, California, U.S.A.).

Hyalella azteca DNA was extracted using the BioRad Aquapure genomic DNA isolation kit (Bio-Rad Laboratories). We amplified a 614 base pair region of CO1 using forward primer 'Jyothi' and a reverse primer modified from HCO 2198 termed ''2198B'' based on preliminary amphipod sequences from universal primers (5′-TTAACTTCAGGGTGACCAAAAAATAC-3′). PCR reactions contained 1.2 μL of 25 mM $MgCl_2$, 2.0 μL 8 μM dNTPs, 2.0 μL of 10x PCR buffer, 0.85 μL of each primer (10 μM), 0.12 μL *Taq* and 2 μL of DNA template. The reaction was adjusted to a total volume of 20 μL by adding sterilized water. DNA was initially denaturated at 94 °C for 2 min, followed by 40 cycles at 94 °C for 30 s, 48 °C for 30 s and a 1-min extension at 72 °C. The cycle ended with a final elongation of 72 °C for 7 min. Successful PCR products were purified and cycle sequenced as described above. Cycle sequenced products were visualized on an ABI Prism 3100 DNA sequencer.

Statistical analysis

Genetic diversity We used the software SEQUENCHER v. 4.1 (Gene Codes Corporation, Ann Arbor, MI, U.S.A.) to edit visually and align 103 *F. quilleri* and 120 *H. azteca* sequences. There were no insertions or deletions in either species. To estimate genetic diversity, we calculated expected heterozygosity H_e (the probability that two randomly chosen copies of a gene will be different alleles) and θ_K (which estimates the population genetic parameter $\theta = n_{e[f]}\mu$ from number of haplotypes K; $n_{e[f]}$ is the female effective population size and μ is the mutation rate). Diversity parameters were calculated for each population of *F. quilleri* and *H. azteca* using the program ARLEQUIN v. 2.0 (Schneider, Roessli & Excoffier, 2000). To test whether there were differences in genetic diversity between populations inhabiting intermittent and perennial streams, we performed two-sample *t*-tests for each species using SYSTAT v.10 (Systat Software, Inc., Richmond Point, CA, U.S.A.).

Genetic structure For both species, we estimated the gene genealogy as a haplotype network constructed using statistical parsimony (Templeton, Crandall & Sing, 1992) with the software TCS v. 1.21 (Clement, Posada & Crandall, 2000). We resolved one ambiguous mutational relationship in the *F. quilleri* network that TCS could not fully resolve using the criteria of Crandall, Templeton & Sing (1994) and Templeton, Routman & Phillips (1995) (see Fig. 3).

We estimated population subdivision using F_{ST} (Wright, 1951) and Φ_{ST} (Excoffier, Smouse & Quattro, 1992). In contrast to F_{ST}, which is calculated using only haplotype frequencies, Φ_{ST} also considers the genetic distances among haplotypes. These statistics were calculated using a hierarchical analysis of molecular variance (AMOVA: Excoffier *et al.*, 1992) using the program ARLEQUIN v. 3.0 (Schneider *et al.*, 2000). Statistical significance was obtained from 1000 random permutations. For the mayfly *F. quilleri*, we assessed whether there was genetic differentiation among all sites (regardless of catchment) and among sites within each catchment by testing the null hypotheses ($F_{ST} = 0$) and ($\Phi_{ST} = 0$). We estimated differentiation among catchments (with sites pooled within catchments) as F_{PT} and Φ_{PT}. We also analysed population genetic differentiation with a single hierarchical model, using differentiation among sites within catchments (F_{SC}, Φ_{SC}) and differentiation among catchments relative to total (F_{CT}, Φ_{CT}).

In the amphipod *H. azteca*, a preliminary analysis of haplotype frequencies showed high levels of divergence between: (i) sites upstream of the Hodges Basin and (ii) sites in Hodges Reservoir, its direct tributaries, and downstream sites. Hodges Basin is adjacent to Hodges Reservoir and below San Pasqual Valley (Fig. 1). Although the Hodges Basin may contain water during the wet season, it is not considered part of the main body of Hodges Reservoir. For clarity, we refer to these two portions of the catchment as the upper and lower ''catchment segments''. We assessed

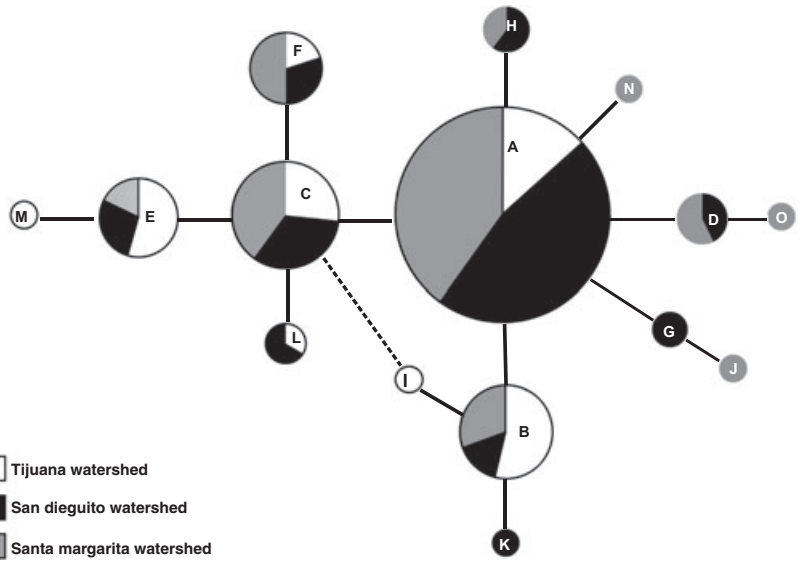

Fig. 3 Haplotype network for *Fallceon quilleri*. Each pie chart represents a single haplotype (A,B,C….O), with the size proportional to the number of individuals who possess that particular haplotype. Solid back lines represent one mutational step. The dashed line represents an ambiguous mutational step in the initial network that was resolved (see *Methods*).

genetic differentiation in *H. azteca* with a hierarchical model that included sites within each stream segment (Φ_{SR}) and differentiation among stream segments (Φ_{RT}). Because the hierarchical model does not separately calculate the degree of differentiation for each stream segment, we conducted additional analyses for the upper and lower segments separately. To evaluate whether directional gene flow in *H. azteca* may affect levels of diversity, we tested for significant differences in genetic diversity (H_e and θ_K) between the upper and lower catchment segments with two sample *t*-tests.

Even without extreme geographical discontinuities in gene flow, limited connectivity among continuously distributed populations will produce patterns of isolation by distance (IBD), where population divergence increases with geographical distance (Wright, 1943). We tested for IBD in *F. quilleri* and *H. azteca* using F_{ST} as a measure of genetic distance. Aerial distance (the shortest distance between two sites) was measured among *F. quilleri* population pairs because adult dispersal in *F. quilleri* is primarily terrestrial. Distance among *H. azteca* populations was estimated following stream contours using USGS 7. 5′ minute series maps in TOPO! v. 2.7.7 (National Geographic Maps, Evergreen, CO, U.S.A.). We used a Mantel test to test the null hypothesis of no association between the matrix of pairwise genetic distances and the geographic distance matrix with the program IBD Web Service v. 2.1 (Jensen, Bohonak & Kelley, 2005). Statistical significance was obtained from 1000 random permutations in each case.

To interpret genetic differentiation in terms of current gene flow requires numerous assumptions, including gene flow/drift equilibrium and that population sizes are not changing (Larson, Wake & Yanev, 1984; Bossart & Prowell, 1998; Bohonak & Roderick, 2001). We tested the null hypothesis that each species' gene genealogy represents a selectively neutral marker at equilibrium using the program FLUCTUATE (Kuhner, Yamato & Felsenstein, 1998), and Fu's *Fs* test (Fu, 1997) as implemented in the software DNASP (Rozas *et al.*, 2003). FLUCTUATE uses a maximum likelihood approach to estimate current θ ($=n_{e[f]}\mu$ as defined above) and the exponential growth or decay rate *g*. Program settings included the Watterson estimate of θ, initial growth rate = 0.1, 10 short chains of 5000 steps, three long chains of 20 000 steps. Fu's *Fs* evaluates the number of haplotypes (*K*) based on π (the mean number of pairwise differences between all sequences). In a growing population, Fu's $Fs < 0$ if the number of haplotypes (*K*) is large relative to π. Although so-called ''neutrality tests'' are sometimes performed across the entire study area, they implicitly assume a single panmictic population. Based on the AMOVA results, we analysed *F. quilleri* with all individuals pooled, and we analysed the upper and lower segments of the San Dieguito catchment separately for *H. azteca*.

Results

Genetic diversity

We found higher genetic diversity in *F. quilleri* than in the *H. azteca* populations that we sampled (Tables 1 & 2). A total of 15 haplotypes were found in 18 *F. quilleri* populations, with four haplotypes common in all catchments and eight haplotypes restricted to one or two catchments (Table 3). In contrast, only four haplotypes were found in the 14 *H. azteca* populations sampled (Table 4). The haplotype network revealed one ancestral haplotype and one derived haplotype in the upper stream segment of the San Dieguito catchment, and one ancestral haplotype and one derived haplotype in the lower stream segment. The two ancestral haplotypes were separated by a 15 base pair difference (Fig. 4).

We failed to detect significant differences between intermittent and perennial streams for genetic diversity in the mayflies (H_e: $t = -0.073$, $P = 0.94$ and θ_K: $t = -0.228$, $P = 0.82$) and in the amphipods (H_e: $t = -0.602$, $P = 0.58$ and θ_K: $t = -0.581$, $P = 0.57$).

Genetic structure

In *F. quilleri*, genetic differentiation among sites was statistically significant using both F_{ST} and Φ_{ST}, regardless of the population model (Table 5). Supplementary analyses demonstrated that this result was due to differentiation between the southernmost catchment (Tijuana) and the other two. Analyses at the level of each population pair showed that Pine Creek in the Tijuana catchment was particularly unusual (Table 6), and the AMOVAS were not significant with this site removed. In *H. azteca*, the AMOVA analyses revealed very strong genetic divergence between the upper and lower segments of the

Table 3 Haplotype distributions for *Fallceon quilleri*

	Tijuana					San Dieguito							Santa Margarita					
Haplotype	PC	COT	LP	WC	CC	SY	BC	HV	KC	GV1	GV2	SDR1	SMRDC	SMR	ST	RBC	SD	DL
A	1		1	2		2	2	2	1	3	1	3		5	2	1	1	3
B		1	2	1	3						1	1	1	1		1	1	
C			1	1	2	1	2					2	2			2	2	
D									1	1		1				2		2
E	4		2	1		1		2					1				1	
F	1	1						2	1				1	1	1			2
G											2							
H							1		1	1			1		1			
I				1														
J																		1
K											1							
L					1		1		1									
M			1															
N															1			
O															1			

Table 4 Haplotype distributions for *Hyalella azteca*

Haplotype	SUTH	TC2	TC1	SYNT1	SYNT2	HV	KCAL	GV1	GV2	HODG1	HODG2	SDRES	SDR1	SDR2
A	8	7	5	9	8	12	7	3		2		2	3	4
B			1	1		1	1	7	9	7	3	5	6	6
C											1		1	
D							1							

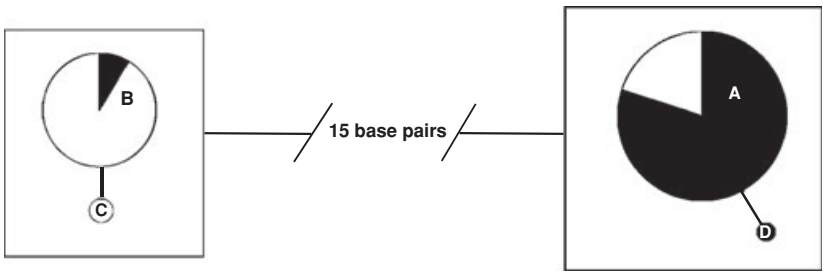

Fig. 4 Haplotype network for *Hyalella azteca* in the San Dieguito catchment. Fifteen base pairs separate the two clades.

catchment, and no genetic divergence among sites within stream segments (Tables 5 & 7). The degree of differentiation between the upper and lower catchment segments ($\Phi_{ST} = 0.661$) was much greater than any of the *F. quilleri* analyses. Expected heterozygosity was significantly greater in the lower catchment segment than in the upper ($t = 2.70$, $P = 0.02$), but θ_K was not ($t = 0.87$, $P = 0.40$).

Analyses of IBD also gave contrasting results for each species. The association between genetic distance and geographical distance in *F. quilleri* was not statistically significant, whether Pine Creek was included ($Z = 636.09$, $r = 0.17$, $P \leq 0.06$) or excluded ($Z = 406.58$, $r = 0.17$, $P \leq 0.07$: see Fig. 5). In *H. azteca*, a highly significant pattern of IBD was detected when all pairwise comparisons were analysed together ($Z = 80.38$, $r = 0.35$, $P \leq 0.006$), although this was due entirely to contrasts between the upper and lower segments of the catchment (Fig. 5).

Fu's *Fs* was significantly less than zero for *F. quilleri* ($Fs = -6.42$, $P = 0.036$), although a cutoff of $P < 0.02$ may be more appropriate for this test than $P < 0.05$ (Fu, 1997). Reflecting the rare, recent mutations radiating from haplotype A (also referred to as a star

Table 5 Analysis of molecular variance (AMOVA) values for *Fallceon quilleri* and *Hyalella azteca*

	Pine Creek included		Pine Creek removed	
F. quilleri	Φ_{ST}	F_{ST}	Φ_{ST}	F_{ST}
Hierarchical Island Model				
Among sites relative to catchment (SC)	0.047*	0.033*	0.022	0.010
Among catchments relative to total (CT)	0.030	0.012	0.019	0.015
Within catchments (ST)				
Tijuana	0.070	0.078	−0.068	−0.031
San Dieguito	0.073	−0.009	0.073	−0.009
Santa Margarita	0.006	0.045	0.006	0.045
Among all sites (ST)	0.067*	0.041*	0.034	0.020
Catchments only (PT)	0.062**	0.043**	0.024	0.017

	San Dieguito Catchment	
H. azteca	Φ_{ST}	F_{ST}
Hierachical Island Model		
Among sites relative to segment (SR)	−0.015	−0.013
Between segments relative to total (RT)	0.661**	0.612**
Upper segment (ST)	−0.062	−0.037
Lower segment (ST)	−0.001	−0.006

*$0.01 < P < 0.05$; **$P < 0.01$.

like phylogeny; Fig. 3), $Fs < 0$ is consistent with recent population growth. In *H. azteca*, *Fs* was positive in both segments of the San Dieguito catchment, reflecting an absence of rare haplotypes (Fig. 4) and suggesting that populations may be shrinking in size (upper: $Fs = 4.58$, $P = 0.038$, lower: $Fs = 13.07$, $P < 0.001$). The FLUCTUATE results also indicated population growth in the mayflies ($\theta = 0.021$ with a SD of 0.0012 $g = 2598$ with a SD of 154.34). However, we did not interpret the estimate of g literally, because it can be strongly biased upwards (Kuhner *et al*., 1998). FLUCTUATE suggested population decline in both the upper ($\theta = 0.002$, $g = -254.14$) and lower ($\theta = 0.002$, $g = -325.24$) stream segments for *H. azteca*. However, g was not significantly different from zero in the amphipods, because low genetic diversity led to extremely high variance in these estimates.

Discussion

Genetic diversity

We found more genetic diversity in the mayfly *F. quilleri* than in the amphipod *H. azteca* both across the same sampling range (San Dieguito catchment) and overall. In this study, although there were only two species sampled, our results are consistent with other studies that have compared genetic diversity in invertebrate species of different dispersal abilities (e.g. Zera, 1981; Myers, Sperling & Resh, 2001; reviewed by Peterson & Denno, 1998 and Bohonak, 1999). Although genetic diversity is a function of effective population size, during collecting efforts we did not notice that these two species had drastically different abundances. This suggests that the gene pool for *F. quilleri* integrates over a much larger geographical area than for *H. azteca*. We failed to find differences in genetic diversity between perennial and intermittent sites in either species. In *F. quilleri*, any such differences (if they do exist) are probably to be overwhelmed by extensive adult dispersal across the wide geographical area included in the gene pool. We hypothesize that in streams which are drying, emerging adults disperse to oviposit in better habitat such as perennial streams. Once flow returns intermittent streams are recolonized by many individuals, possibly from more than one source population. Aquatic insects in arid regions have asynchronous emergence that make local extinction less likely (reviewed by Mackay, 1992). Additionally, previous studies have indicated that flying adult mayflies can sense optimal ovipositing habitat. For example, Peckarsky, Taylor & Caudill (2000) demonstrated that adult *Baetis bicaudatus* (Dodds) disperse from their natal stream to

Table 6 *Fallceon quilleri* pairwise aerial distances (km) (above diagonal) and Φ_{ST} values (below diagonal)

	PC	COT	LP	WC	CC	SY	BC	HV	KC	GV1	GV2	SDR1	SMRDC	SMR	ST	RBC	SD	DL
PC	–	8.74	16.24	22.37	26.10	34.59	43.65	52.02	55.14	55.88	55.03	60.10	91.01	90.31	89.44	89.31	92.57	99.06
COT	−0.083	–	7.82	22.64	17.96	43.21	52.06	59.78	62.82	63.35	62.52	67.37	99.69	98.97	98.05	97.87	101.10	107.51
LP	0.006	−0.020	–	22.68	10.17	50.83	58.48	65.27	68.21	68.46	67.66	72.23	106.58	105.80	104.77	104.44	107.62	113.88
WC	0.333*	−0.215	−0.020	–	25.49	47.17	46.21	49.08	51.52	51.00	50.32	53.98	94.52	93.45	91.91	90.91	93.76	99.30
CC	0.317	−0.215	0.008	−0.175	–	60.57	66.72	72.35	75.11	75.04	74.29	78.46	115.20	114.34	113.18	112.65	115.74	121.79
SY	0.052	−0.030	−0.127	−0.030	0.034	–	20.81	33.85	36.87	39.28	38.45	44.09	47.86	58.67	58.38	59.05	62.51	69.58
BC	0.260	0.023	0.056	−0.001	0.031	−0.126	–	13.07	16.05	18.52	17.72	23.30	48.64	47.70	46.46	45.97	49.13	55.46
HV	−0.050	−0.119	−0.024	0.119	0.145	−0.120	0.044	–	3.27	5.46	4.68	10.24	47.12	45.80	43.86	42.39	45.02	50.27
KC	0.216	−0.178	0.060	−0.012	0.024	−0.102	−0.147	−0.012	–	3.06	2.70	7.36	45.80	44.40	42.32	40.64	43.11	48.08
GV1	0.509*	0.242	0.232	0.167	0.242	0.156	0.105	0.294*	−0.042	–	0.89	4.84	47.78	46.33	44.13	42.26	44.58	49.22
GV2	0.480*	0.027	0.197	0.054	0.114	0.200	0.223*	0.314*	0.136	0.143	–	5.71	48.04	46.60	44.44	42.63	45.00	49.73
SDR1	0.364*	0.013	0.073	−0.089	0.020	−0.063	−0.032	0.138	−0.072	−0.019	0.106	–	47.86	46.30	43.90	41.70	43.71	47.75
SMRDC	0.048	−0.227	−0.071	−0.053	−0.020	−0.156	−0.085	−0.114	−0.087	0.184	0.209*	0.020	–	1.80	4.77	8.30	9.70	15.47
SMR	0.437*	0.007	0.143	−0.018	0.096	0.042	0.056	0.177	−0.021	−0.002	0.086	−0.085	0.088	–	2.97	6.52	8.18	14.39
ST	0.314*	−0.068	0.128	0.031	0.117	−0.024	−0.014	0.100	−0.124	−0.119	0.096*	−0.059	0.033	−0.062	–	3.60	5.86	12.78
RBC	0.331*	−0.034	0.070	−0.057	0.022	−0.035	0.000	0.129	−0.095	−0.010	0.120	−0.139	0.020	−0.002	−0.055	–	3.51	10.79
SD	0.074	−0.141	−0.142	−0.121	−0.075	−0.226	−0.079	−0.074	−0.047	0.214	0.185	−0.036	−0.171	0.076	0.049	−0.024	–	7.30
DL	0.322*	−0.097	0.174*	0.098	0.170*	0.040	0.075	0.073	−0.088	0.032	0.145	0.005	0.067	−0.006	−0.062	−0.018	0.098	–

*$P \leq 0.05$.

Table 7 *Hyalella azteca* pairwise catchment distances (km) (above diagonal) and Φ_{ST} values (below diagonal)

	SUTH	TC01	TC02	SYNT1	SYNT2	HV	KC	GV1	GV2	HODG1	HODG2	SDRES	SDR1	SDR2
SUTH	–	11.24	9.27	20.23	20.19	32.89	38.10	40.75	40.17	43.33	40.37	48.8	49.85	55.39
TC1	0.051	–	1.97	8.99	8.95	21.65	26.86	29.51	28.93	32.09	29.13	37.56	38.6	44.15
TC2	0.000	0.028	–	10.96	10.92	23.62	28.83	31.48	30.90	40.57	31.10	39.53	40.57	46.12
SYNT1	−0.024	−0.130	−0.040	–	0.04	12.66	17.87	20.52	19.94	23.10	20.14	36.14	29.65	35.2
SYNT2	0.000	0.051	0.000	−0.024	–	12.70	17.91	20.56	19.98	23.14	20.18	36.1	29.61	35.16
HV	−0.042	−0.090	−0.055	−0.093	−0.042	–	5.21	7.86	7.28	10.44	7.48	15.91	16.95	22.5
KC	−0.014	−0.142	−0.031	−0.112	−0.014	−0.091	–	4.88	4.19	7.65	4.81	15.63	15.67	18.22
GV1	0.637*	0.353	0.620*	0.495*	0.637*	0.558*	0.463	–	1.45	7.85	1.21	8.12	9.23	15.41
GV2	1.000*	0.841*	1.000*	0.883*	1.000*	0.901*	0.867*	0.206	–	6.91	1.99	8.17	9.47	15.52
HODG1	0.736*	0.467*	0.722*	0.598*	0.737*	0.654*	0.567*	−0.101	0.125	-	7.90	3.13	4.36	10.58
HODG2	0.989*	0.841*	1.000*	0.883*	1.000*	0.901*	0.867*	0.206	0.217	0.125	-	6.88	8.02	14.9
SDRES	0.686*	0.365*	0.667*	0.522*	0.686*	0.591*	0.486*	−0.138	0.214	−0.133	0.061	–	1.08	7.56
SDR1	0.630*	0.348	0.612*	0.490*	0.630*	0.552*	0.458*	−0.109	0.200	−0.099	0.068	−0.136	–	6.35
SDR2	0.521*	0.215	0.501*	0.368	0.521*	0.437*	0.335*	−0.087	0.316	−0.039	0.185	−0.106	−0.086	–

*$P \leq 0.05$.

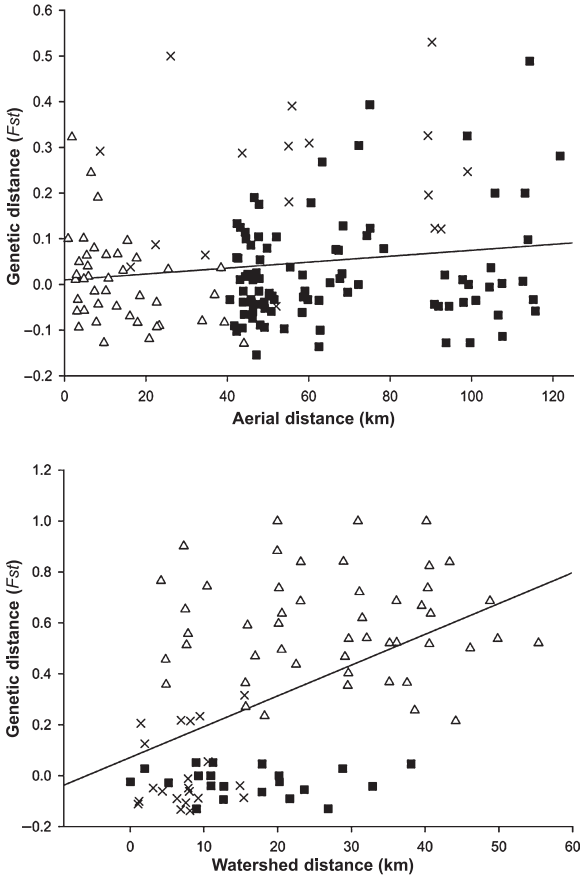

Fig. 5 Isolation by distance plots for *Fallceon quilleri* (top) and *Hyalella azteca* (bottom). For the *F. quilleri* plot, open triangles indicate population pairs in the same catchment, squares indicate population pairs in different catchments, and crosses show population pairs that include Pine Creek. For *H. azteca*, squares indicate population pairs in the upper catchment segment of the San Dieguito catchment, crosses represent population pairs in the lower catchment segment, and open triangles represent population pairs across catchment segments.

oviposit if local habitat conditions are not optimal. Optimal being defined as partially exposed rocks under which females could crawl to oviposit. Furthermore, Monaghan *et al.* (2001) found that, while in flight, *Baetis alpinus* (Pictet) that encountered a lake immediately stopped to oviposit in flowing water below the lake. Thus, it may be that *F. quilleri* responds to cues such as stream flow when choosing oviposition sites to maximize offspring survival.

The amphipod *H. azteca* is less diverse genetically than *F. quilleri*. In addition, only one rare allele was detected in each of the catchment segments. A standard interpretation for such patterns is that effective population size is (or has recently been) shrinking. However, population sizes in the catchment are probably greater now than historically, when seasonal drying and flooding events were the norm. Most perennial streams in the San Dieguito catchment were historically intermittent with urban runoff and reservoirs built within in the last 100 years (Haelsig, 1964). Low genetic diversity and death of rare alleles may be better explained by the vulnerability of obligately aquatic amphipods to seasonal drying and flooding events. Dramatic fluctuations in local population size and routine extinctions can accelerate genetic drift and skew allele distributions. It is unclear how much of the current diversity in *H. azteca* represent historic conditions or the current mix of intermittent and permanent habitats. We initially predicted that intermittent sites would have less genetic diversity than perennial sites. There was no statistical support for this hypothesis, however, probably because of low statistical power. Further analyses with more variable genetic markers may resolve this discrepancy.

Genetic structure

In this study, although we used only two species, analyses on genetic structure gave quite contrasting results for *F. quilleri* and *H. azteca*. Our results suggest that in *F. quilleri* dispersal is widespread, and that new colonists of sites are genetically diverse, the latter probably being because of oviposition by many individuals and/or several source populations. However, we did not detect genetic differentiation at the largest spatial scale, and genetic structure in San Diego County did not follow any clear model of evolution. For example, there was no IBD, which Miller *et al.* (2002) demonstrated as accurately reflecting dispersal ability in some species of stream-dwelling invertebrates, and patterns of genetic differentiation did not reflect stream hierarchies as in other invertebrates (Hughes *et al.*, 1999) and fishes (Meffe & Vrijenhoek, 1988). The haplotype network for the mayfly possessed a statistically significant excess of rare alleles, which is often interpreted in terms of population growth. Thus, it is possible that this species has recently expanded its range into this region, and patterns of differentiation are approaching a new equilibrium as differentiation increases (Bohonak & Roderick, 2001). However, genetic patterns in this species could also reflect frequent gene

flow across an area even larger than the area we sampled. (*Fallceon quilleri* has a wide distribution across North America and Central America). Because of the limited inferential power from the mtDNA (it represents only one marker with maternal inheritance), tests of these hypothesis would also require additional studies with new markers.

Pine Creek contributed most significantly to genetic differentiation in *F. quilleri*. This site has the highest altitude of any sampled (*c.* 1150 m) and is surrounded by the Laguna Mountains (>1500 m), except for the valley through which the creek itself flows. Colonization to Pine Creek is thus likely to occur from the southwest, as flight over the Laguna Mountains to the north and east would be difficult. We sampled other streams at higher altitudes in the Tijuana catchment, but *F. quilleri* was either absent or extremely scarce. This suggests that dispersal to these sites over the Laguna Mountains is limited or that Pine Creek represents the limit of tolerance by *F. quilleri* for high altitude and/or its abiotic conditions (Rahbek, 1995). Interestingly, the most common haplotype at Pine Creek was also found in La Posta Creek. La Posta Creek is the only permanent stream in the Tijuana catchment and might be the only feasible source of colonists for Pine Creek. However, it is also possible that the unusual genetic composition of Pine Creek is not permanent if this site is recolonized after each dry season by an atypical or highly variable group of colonists. Gibbs *et al*. (1998) suggested such a hypothesis for the mayfly *Siphlonisca aerodromia* (Needham) based on samples from several seasons. Additional samples of *F. quilleri* from this site and others at the edge of its ecological limits would be useful.

In contrast, the amphipod *H. azteca* showed a well-defined pattern of genetic structure at a much smaller geographical scale. Limited dispersal between the upper and lower segments of the San Dieguito catchment is the most obvious inference, with the Hodges Basin and the San Pasqual Valley as a barrier. The San Pasqual Valley is an agricultural region and, although it contains one of the most productive groundwater basins in the San Dieguito catchment, there is rarely surface flow. In typical years, water draining from the upper region of the catchment becomes groundwater recharge in this area. In very wet years, surface flow can occur from Santa Ysabel Creek into the San Dieguito River, which then empties into Hodges Reservoir, making downstream gene flow possible (MacLaggan, 1987). We note that there are higher levels of genetic diversity in the lower than the upper catchment segment although gene flow through the San Pasqual is not frequent enough to prevent strong differentiation between the catchment segments. Similarily, Thomas, Blinn & Keim (1998) noted higher differentiation among populations of *H. azteca* living in xeric than among those in mesic environments, presumably because of reduced gene flow. Within the upper and lower catchment segments, we did not find significant genetic divergence among populations. Each segment contains permanent streams and reservoirs that may be source populations for streams that undergo extinctions.

Strangely, a majority of the amphipods sampled from Kit Carson Creek and Highland Valley Creek in the lower portion of the catchment possessed the ancestral haplotype that predominates in the upper segment (Fig. 2). These represent the eastern-most sites in the lower segment, flowing into Hodges Basin and Hodges Reservoir and hydrologically isolated from the upper segment. Because current patterns of connectivity provide no explanation for this pattern, it may reflect connectivity before anthropogenic land-use changes. San Pasqual Valley is used for agriculture and water flowing into the region has previously been diverted via irrigation canals. The extensive diversion of groundwater in the valley for irrigation suggests that historical surface flow may have facilitated some seasonal gene flow among sites above and below the valley.

Sequence divergence between ancestral *H. azteca*, haplotypes A and B was high (2.5%; 15 extinct alleles estimated). Studies examining cryptic species complexes of *Hyalella* have demonstrated sequence divergence in cytochrome oxidase 1 as high as 20% (Witt, Blinn & Hebert, 2003), suggesting that the two segments of the catchment do not represent different species. The agricultural conversion of the San Pasqual Valley may also be too recent to explain this degree of divergence, although more data on effective population sizes and mutation rates in *H. azteca* are needed to evaluate this hypothesis. Alternatively, we note that Hodges Reservoir is largely supplied with water that is imported from the Colorado River. However, Sutherland Reservoir (in the upper segment of the catchment) is maintained only by natural

runoff. Thus, an alternative hypothesis is that aqueducts have transported *H. azteca* from the Colorado River into Hodges Reservoir, and these genotypes have replaced the 'native' genotypes in the lower catchment. In the current data set, haplotypes B and C are common only in the lower catchment in sites directly connected to Hodges Reservoir by permanent flow (in contrast to Kit Carson Creek and Highland Valley Creek).

In this study we found that genetic structure and diversity in coastal southern California stream and reservoir invertebrates are shaped more by dispersal ability than by seasonal drying and flooding events. Nonetheless, in the amphipod *H. azteca* there is very little gene flow between the upper and lower sections of the catchment sampled. High levels of sequence divergence in *H. azteca* may be attributed to recent agricultural modifications to the catchment, or possibly imported drinking water. Permanent water sources, including reservoirs, appear to promote gene flow among populations that were likely to have been isolated historically, or are unable to support amphipods at all. The mayfly *F. quilleri* is genetically homogeneous throughout the region, although definitive conclusions about the roles of population history and long distance gene flow will require additional studies. In general, this study suggests that, in areas where streams are currently impacted by urbanization or have the potential to be impacted, alterations to water flow and connectivity appear to have greater potential to impact the genetic structure and diversity in species that are restricted to the aquatic environment than those with overland dispersal abilities.

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