Genetic diversity in the *Gammarus pecos* species complex: Implications for conservation and regional biogeography in the Chihuahuan Desert

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Abstract

We used allozyme electrophoresis to quantify genetic variation in nine populations of the *Gammarus pecos* species complex endemic to spring systems of the northern Chihuahuan Desert. There was significant within-population and high among-population genetic variation. Two populations exhibited heterozygote deficiencies and high proportions of polymorphic loci, which suggests the presence of cryptic species. Genetic distances among populations were negatively correlated with previously published morphological similarities, which suggests congruence between allozyme and morphological phenotypes. Cluster analysis of genetic distances showed four major groups of populations within the *G. pecos* complex. Genetic identities and fixed allelic differences support the presence of at least four distinct species: *Gammarus desperatus, G. pecos, Gammarus hyalleloides,* and one or more undescribed species. Relatively large genetic distances between populations suggest long periods of isolation and allopatric speciation. Patterns of among-population genetic variation were similar between amphipods and several groups of endemic fishes and snails, which suggests a coherence to biogeographic patterns within this region. Thus, the understanding of the genetic structure and taxonomic status of the *G. pecos* species complex provides insight into the biogeography of other aquatic organisms in the northern Chihuahuan Desert. Given the alarming rate at which desert spring systems are being altered and the unique biotic assemblages present, protection of these habitats is imperative.

In desert regions, springs have long been considered to be unlimited sources of water for a variety of human activities. The habitat destruction resulting from such activities is an especially severe problem when it affects regions of high endemism, where most endangered species are found (Dob-

son et al. 1997). The continuing loss of spring habitats and resulting effects on the endemic species occupying them are now recognized as important factors threatening biodiversity in North American deserts (Minckley and Unmack 2000). The development of effective conservation strategies for species at risk requires an understanding of the relationships among geographically separated populations. In particular, it is important to know whether such populations are genetically distinct and therefore unique management units of evolutionarily important lineages (Moritz 1994). Historical isolation and responses to past climatic and geological changes are often reflected in the geographic distribution of genetically distinct populations (Kelt and Brown 2000). Geographic isolation and lack of gene flow among populations of non-vagile species make desert springs an appropriate habitat for studying the evolution and geographical patterns of endemic populations (Myers et al. 2001).

The Chihuahuan Desert of North America is one of the World Wildlife Fund's Global 200 ecoregions because of the uniqueness of its fauna and flora and the significant number of endemic species present (Olson and Dinerstein 1998). It
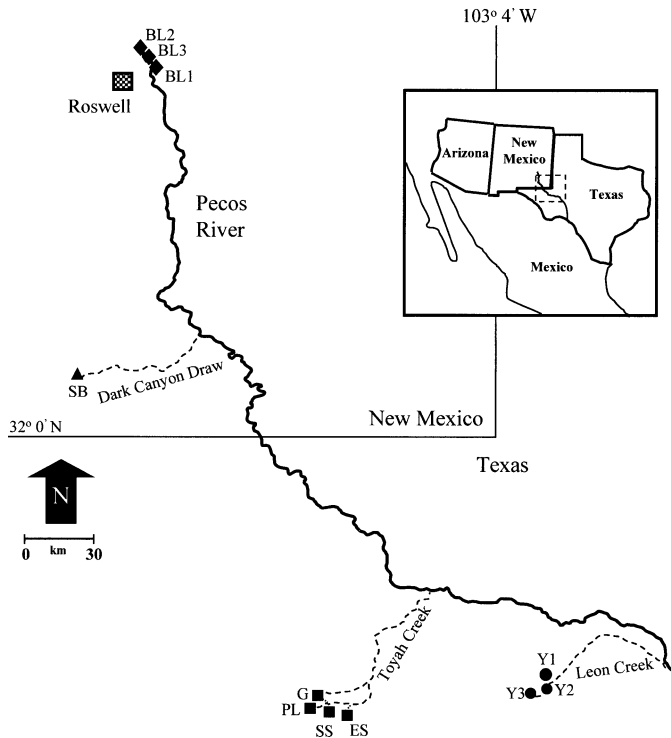
Fig. 1. Collection sites for *G. pecos* complex populations from the Chihuahuan Desert. Population codes are found in Table 1.

stretches from the Rio Grande Valley in southern Mexico to an area just north of Mexico City (Fig. 1). From the Cambrian Era (570 million years ago [mya]) to the Late Cretaceous (~66 mya), this region was covered by a shallow ocean that extended northwest from the Gulf of Mexico. During the Late Cretaceous period the region uplifted, causing the ocean to recede and swamplands to develop (Bousfield 1958; Holsinger 1972). Later drying of this region led to the formation of isolated aquatic habitats scattered throughout the desert. The adaptive processes that local species underwent during these drastic environmental changes have created isolated communities with high proportions of endemic species.

The *Gammarus pecos* species complex consists of endemic amphipod species restricted to isolated spring systems in the northern part of the Chihuahuan Desert (Cole 1985). Members of the complex inhabit a small number of streams, ponds, ditches, sloughs, and springs (Lang et al. 2003) that contain euryhaline, sulfatochloride waters derived ultimately from Permian marine sediments. In all likelihood, this species complex was derived from a common ancestor that was once widespread in the Permian Sea (Bousfield 1958; Holsinger 1972). When the sea level fell during the Late Cretaceous period, populations of this marine amphipod were trapped and isolated in the remaining pools. Genetic drift caused by isolation, along with environmental variation during climatological changes that ensued during the late Pleistocene to early Holocene eras, probably promoted differentiation of populations through allopatric speciation.

Currently, the *G. pecos* species complex is composed of three described species: *G. pecos* Cole and Bousfield (1970),

Gammarus hyalelloides Cole (1976), and *Gammarus desperatus* Cole (1981). *G. pecos* was described from Diamond Y Spring and its effluents in Pecos County, Texas (Cole and Bousfield 1970) and was also reported from San Solomon Spring in Reeves County, Texas (Cole 1985). *G. hyalelloides* was named from specimens in the *Chara* bed at the mouth of Phantom Lake Spring, Jeff Davis County, Texas, ~85 km west of Diamond Y Spring (Cole 1976). *G. desperatus* is apparently confined to a small spring system in Roswell, Chaves County, New Mexico (Cole 1981). In 1985, Cole conducted a morphological study on seven populations of the *G. pecos* complex. The percentage of similarities from Mann-Whitney *U*-tests for 20 morphological traits showed the existence of two morphotypes of undetermined taxonomic affinity (Cole 1985); Cole called these populations "*E*" from Carlsbad, Eddy County, New Mexico, and "*C*" from the canal system of Phantom Lake Spring (Cole 1985).

Surveys during the 1950s found gammarid amphipods to be the most abundant metazoans in the Pecos River drainage of New Mexico and Texas, with average densities as high as 10,416 m^{-2} (Noel 1954). Since the late 1950s, an alarming decline has been observed in the number of extant populations of the *G. pecos* complex. During the early 1960s, the Lander Springbrook population (Chaves County, New Mexico) was extirpated (Cole 1981). No gammarid amphipods were observed in the lateral canal of Phantom Lake Spring during inventories we conducted in March 2000 and June 2001. Since then, the canals have remained dry; thus, it appears that the endemic, undescribed population "*C*" that Cole (1985) reported from the canals is probably extinct.

Habitat loss and alteration (e.g., artesian spring source diversion, dewatering, and capping) and groundwater depletion are considered to be the principal causes of decline in the *G. pecos* species complex (Cole 1981, 1985; Lang et al. 2003). Regional groundwater pumping and oil and gas industry operations are ongoing in the Pecos River Valley. These processes were largely responsible for the extinction of two isolated populations of *G. desperatus* in New Mexico (Cole 1981, 1985).

The decline of Chihuahuan Desert habitats and their associated fauna and flora is of considerable concern to conservation organizations. The development of effective conservation measures requires an understanding of the taxonomic affinities of endemic species and species complexes. In the present article, we describe the population genetic structure of the *G. pecos* species complex, consider the taxonomic affinities of described and undescribed species within the complex, and place the complex within the context of regional patterns of genetic diversity in the northern Chihuahuan Desert.

Methods

Study areas—All known populations of the *G. pecos* complex are in New Mexico and Texas, localized within 100 km of the Pecos River. The most northern populations are those of *G. desperatus* in Bitter Lake National Wildlife Refuge (BLNWR), Chaves County, New Mexico (Cole 1985), and the complex reaches its southern limits in Reeves and Pecos counties, Texas (Fig. 1).

Table 1. Sample site locations and population codes for the *G. pecos* species complex. All individuals were collected within 300 m of the coordinates shown.

Sample site location	Population code	Latitude and longitude
Phantom Lake Spring, Toyah Creek, TX	PL	30°56′05.87″N, 103°50′58.29″W
San Soloman Spring, Toyah Creek, TX	SS	30°56′40.27″N, 103°47′08.42″W
Giffin Spring, Toyah Creek, TX	G	30°56′44.82″N, 103°47′23.26″W
East Sandia Spring, Toyah Creek, TX	ES	30°59′28.10″N, 103°43′43.53″W
Diamond Y Spring, Diamond Y Draw, ''John's hole,'' TX	Y1	31°02′11.49″N, 102°53′28.17″W
Diamond Y Spring, Diamond Y Draw, ''Euphrasia,'' TX	Y2	31°01′56.44″N, 102°53′39.91″W
Diamond Y Spring, Diamond Y Draw, Flume, TX	Y3	31°00′05.37″N, 102°55′26.58″W
Sitting Bull Spring, Lincoln National Forest, NM	SB	32°14′23.11″N, 104°42′01.32″W
Unit 6, Bitter Lake National Wildlife Refuge, NM	BL1	33°26′45.73″N, 104°24′16.30″W
Bitter Creek, Bitter Lake National Wildlife Refuge, NM	BL2	33°28′46.41″N, 104°25′38.65″W
Sago Spring, Bitter Lake National Wildlife Refuge, NM	BL3	33°28′41.34″N, 104°25′11.04″W

BLNWR is located in the floodplain of the Pecos River near Roswell, Chaves County, New Mexico. The refuge contains springs, sinkholes, and marshes. Infrequent inundations of the floodplain, and consequently, periodic mixing of the aquatic populations, were reported until the 1950s when the Pecos River flow was reduced by human water use (Echelle et al. 1987).

Sitting Bull Spring is located in Lincoln National Forest, in the Guadalupe Mountains, Eddy County, New Mexico. This region was once part of the Capitan Barrier Reef; it is now composed of numerous limestone canyons. The spring drains to Sitting Bull Falls, a 39-m waterfall over one of the limestone cliffs. The sampling site is located along Forest Trail 68A upstream of the falls.

Phantom Lake Spring, San Solomon Spring, Giffin Spring, and East Sandia Spring occur in the Toyah Creek basin of Reeves County, Texas, and are drained by tributaries with only intermittent connection to the Pecos River. There are three main artesian springs: Phantom Lake Spring (on land owned by the U.S. Bureau of Reclamation), San Solomon Spring (in Balmorhea State Park), and Giffin Spring (privately owned), plus a few smaller springs, like East Sandia Spring (on land owned by The Nature Conservancy [TNC]). The water flowing out of these springs is used for agricultural irrigation and the springheads and flow paths have been modified by humans for some time. The proximity of the springs suggests that natural surface hydrological connections probably occurred in the past, with the exception of Phantom Lake Spring (the uppermost of the springs), which apparently had no natural outflow stream (Brune 1981). The construction of numerous irrigation ditches connected all of the springs. During the past two decades, diminished flows have again disconnected Phantom Lake Spring from the downstream irrigation system (Sharp et al. 2003). Under the assumption of aquatic dispersal, the potential gene flow should be unidirectional from Phantom Lake Spring to San Solomon Spring to Giffin Spring to Lake Balmorhea and East Sandia Spring (Echelle et al. 1987, 1989).

Diamond Y Spring is drained by Diamond Y Draw, ~16 km north of Fort Stockton, Pecos County, Texas. Diamond Y Spring is a 600-ha preserve owned by TNC since 1990. Gas extraction occurred prior to TNC ownership, with several gas wells located within the preserve borders. *G. pecos* were found at three different sites sampled in the preserve,

all located <3.5 km from each other and having obvious surface flow connections. Since the conclusion of this project, an additional population of *Gammarus* of unknown taxonomic affinity has been discovered at Caroline Spring, Terrell County, Texas.

Populations—These seven spring systems were surveyed during May–June and November 2001. Eleven populations of *Gammarus* were sampled from these systems (Table 1, Fig. 1). These include three populations of *G. desperatus* from BLNWR, New Mexico; *G. pecos* from three locations at Diamond Y Spring, Texas, and one location at San Solomon Spring, Texas; *G. hyalleloides* from Phantom Lake Spring, Texas; and undescribed populations from Sitting Bull Spring, New Mexico, Giffin Spring, Texas, and East Sandia Spring, Texas (Table 1). At least 50 amphipods from each population of the *G. pecos* complex were collected. In addition, individuals of two other gammarid taxa (*Gammarus fasciatus* and *Echinogammarus ischnus;* 50+ individuals per taxon) were collected from the western basin of Lake Erie at Put-in-Bay, Ohio, and used as outgroups.

Sample collection—In May–June 2001, we were able to collect amphipods from all sites except Phantom Lake Spring. Amphipods were collected during a return trip to the latter site in November 2001. At each site, we recorded latitude and longitude using global positioning satellite receivers (Table 1) and collected amphipods with sweep nets and by hand. Amphipods were most abundant beneath calcareous rocks. Amphipods were stored live in plastic bags (~25 individuals per bag) and kept in a cooler until they were individually stored in centrifuge vials and flash-frozen in liquid nitrogen. Samples were stored at −80°C until analysis.

Analytical techniques—Genetic variation within and among populations was analyzed by estimating allozyme variation using cellulose acetate electrophoresis following standard methods (Hebert and Beaton 1989) with modifications. Eight enzyme systems were used to reveal a total of 11 putative loci (esterase, *EST,* enzyme number 3.1.1.1; aspartate aminotransferase, *AAT,* 2.6.1.1; glucose-6-phosphate isomerase, *GPI,* 5.3.1.9; lactate dehydrogenase, *LDH,* 1.1.1.27; malate dehydrogenase, *MDH,* 1.1.1.37; malic enzyme, *ME,* 1.1.1.40; mannose phosphate isomerase, *MPI,*

5.3.1.8; phosphoglucomutase, *PGM,* 2.7.5.1). Each individual was homogenized with 28 μl of 2% 2-phenoxyethanol and then centrifuged at $14,000 \times g$ for 3 min. Cellulose acetate plates (Helena Laboratories) were loaded with the supernatant solution and run at 210 V or 20 mA for 40–80 min, depending on the buffer used (tris-glycine [pH 8.5] for *GPI* and *PGM*; phosphate [pH 8.0] for all other systems) and the specific enzyme. All enzyme systems were scored for a single locus, except *AAT, LDH,* and *MDH,* which were scored at 2 loci each. Alleles were identified by assigning 1 to the allele that migrated furthest anodally (the fastest allele) and 2, 3, etc. to the second fastest, third fastest, etc.

Analyses of the electrophoretic results were conducted using BIOSYS-1 (Swofford and Selander 1981) and Tools for Population Genetic Analysis (TFPGA; Miller 1997). Descriptive measures of genetic variation calculated within populations included (1) average numbers of alleles per locus, (2) allele frequency, (3) number of rare alleles (i.e., alleles with frequency ≤ 0.01), (4) number of unique alleles (i.e., alleles present in only one population), (5) genotype frequency, (6) percentage of polymorphic loci (i.e., loci with the most common allele frequency ≤ 0.95), and (7) mean direct-count heterozygosity per locus (H). We tested for significant differences in mean heterozygosity among populations by performing a one-way analysis of variance (ANOVA) followed by a Bonferroni multiple-comparison test. Agreement of genotype frequencies with Hardy-Weinberg expectations was tested with exact tests of goodness-of-fit. A sequential-comparison Bonferroni technique was used to minimize type 1 error (Lessios 1992).

Along-river and overland geographic distances among populations were measured using digital topographic maps in Arc-View (Environmental Systems Research Institute). The distances reported are the averages of three measurements performed on the maps. Nei (1978) unbiased genetic distance (D) was calculated using TFPGA (Miller 1997). Mantel tests were then performed between the geographic distances and the genetic distances among populations to evaluate the correlation between the two measures as a test for isolation-by-distance. Data were log transformed, because untransformed scatter plots of genetic distance versus geographic distance were nonlinear.

Exact tests and Weir and Cockerham's (1984) method of calculating Wright's (1978) *F*-statistics, as well as D and Nei (1978) unbiased genetic identity (I), were used to estimate genetic variation among populations. The exact tests to determine whether significant differences in allele frequencies existed among populations (Raymond and Rousset 1995) were followed by a sequential-comparison Bonferroni technique to minimize type 1 error (Lessios 1992). To estimate gene flow between populations, we calculated the number of migrants exchanged between populations per generation (Nm) by solving the equation $Nm = (1 - \theta)/(4\theta)$ (Slatkin and Barton 1989), where θ is Weir and Cockerham's (1984) analog to Wright's (1978) F_{ST}.

Using the average genetic distance between the described species in the *G. pecos* species complex and data from the literature (Thorpe 1982; Stewart 1993) as criteria for species boundaries, the presence of possible distinct taxonomic species (i.e., populations with greater genetic distance than the average genetic distance between described species) was tested. Mantel tests between D and Cole's (1985) morphological similarities were performed to evaluate the possibility of distinct taxonomic species (i.e., a strong negative correlation between the two matrices is evidence that the genetic data are consistent with Cole's morphological data; therefore, it is considered additional evidence that Cole's undetermined morphotypes might be distinct species). Because his values for morphological similarity were percentages, Cole's (1985) morphological data were arcsine-transformed before the Mantel test was performed.

Nei (1978) unbiased genetic distances between all pairs of amphipod populations, including those from Lake Erie, were used to construct a phenogram using the unweighted-pair-group method with arithmetic averaging (UPGMA; Swofford and Olsen 1990). Confidence in the nodes of the phenogram was assessed by bootstrapping 1,000 replicates.

Results

Within population genetic variation—Mean sample size per locus was 33.8 individuals for the *G. pecos* species complex populations, and slightly smaller for the outgroup populations (Table 2). Small sizes of individuals limited us to a single run of each enzyme system and, as a result, we were not able to score all loci for all individuals. Thus, sample sizes vary for each locus-by-population combination.

Allele frequencies varied among populations. Bitter Lake populations shared the same predominant allele at most loci (Table 2). Predominant alleles were also similar among Toyah Creek populations and among Diamond Y Spring populations (Table 2; site codes given in Table 1). Rare alleles were found in populations BL2, ES, and SS (Table 2). Unique alleles were found in populations BL1, ES, PL, and SB (Table 2). The *G. pecos* species complex had the same average number of alleles per locus as *G. fasciatus,* and both were higher than *E. ischnus*. In general, populations of the *G. pecos* species complex showed higher levels of polymorphism than the Lake Erie samples. The ANOVA comparing mean heterozygosity per individual for the 13 populations was significant ($P < 0.001$). The Bonferroni multiple comparison test (experimentwise error rate $\alpha = 0.05$) showed that heterozygosity in *G. fasciatus* and *E. ischnus* was significantly lower than in the *G. pecos* species complex populations. Within the *G. pecos* complex, only populations G and SB were significantly different from each other.

Comparison of the observed genotype frequencies with Hardy-Weinberg (HW) expected frequencies showed that all of the studied populations had at least two loci significantly different from HW expectations, except for Diamond Y Spring populations (Table 2; genotypic data available by request to D.J.B.). Overall, 27.3% of locus-by-population combinations at polymorphic loci for the *G. pecos* species complex had genotype frequencies different than HW expectation; all were heterozygote deficiencies (Table 2).

Among population genetic variation—Exact tests performed on allele frequencies of the Diamond Y Spring populations showed no significant heterogeneity among them (data not shown). The three Diamond Y Spring samples were

Table 2. Allele frequencies at polymorphic loci for gammarid amphipods from the Chihuahuan Desert and Lake Erie. Population codes are given in Table 1. Numbers in parentheses are standard errors.

Locus and allele	ES	SS	G	PL	Y1	Y2	Y3	SB	BL1	BL2	BL3	G. fasciatus	E. ischnus
PGM	$n = 30$	$n = 45^*$	$n = 35$	$n = 16^*$	$n = 11$	$n = 10$	$n = 7$	$n = 3$	$n = 35$	$n = 36$	$n = 41^*$	$n = 26^*$	$n = 28$
1	0.017	—	—	0.500	—	—	—	—	0.029	—	—	—	—
2	0.017	—	0.029	0.031	—	—	—	1.000	0.029	0.028	0.012	0.096	1.000
3	0.050	0.067	0.700	0.188	—	—	—	—	0.114	0.833	0.256	0.865	—
4	0.700	0.433	0.186	0.281	1.000	0.850	1.000	—	0.257	0.139	0.646	0.038	—
5	0.217	0.444	0.071	—	—	0.150	—	—	0.557	—	0.085	—	—
6	—	0.056	0.014	—	—	—	—	—	0.014	—	—	—	—
MPI	$n = 48^*$	$n = 60^*$	$n = 43^*$	$n = 46^*$	$n = 20^*$	$n = 11$	$n = 11$	$n = 38^*$	$n = 39^*$	$n = 52$	$n = 44^*$	$n = 31^*$	$n = 29^*$
1	—	—	—	—	—	—	—	—	—	—	—	0.065	0.879
2	—	—	—	—	—	—	—	—	—	—	—	0.661	0.034
3	0.021	0.017	0.023	—	—	0.045	—	—	—	—	—	0.274	0.086
4	0.677	0.325	0.651	0.402	0.225	0.500	0.045	0.158	0.141	0.038	0.250	—	—
5	0.302	0.650	0.047	0.522	0.725	0.455	0.864	0.842	0.859	0.962	0.750	—	—
6	—	0.008	0.279	0.076	0.050	—	0.091	—	—	—	—	—	—
EST	$n = 49^*$	$n = 60^*$	$n = 43^*$	$n = 44^*$	$n = 20$	$n = 19^*$	$n = 11$	$n = 38^*$	$n = 38^*$	$n = 52^*$	$n = 44$	$n = 31^*$	$n = 30^*$
1	0.286	0.358	0.023	—	0.125	0.105	0.045	0.829	0.395	0.010	—	0.032	—
2	0.469	0.433	0.093	0.068	0.075	0.079	—	0.171	0.316	0.163	0.273	0.016	—
3	0.235	0.150	0.616	0.841	0.250	0.421	0.091	—	0.197	0.692	0.727	0.661	0.083
4	—	0.058	0.256	0.091	0.525	0.342	0.864	—	0.092	0.135	—	0.242	0.917
5	—	—	0.012	—	0.025	0.053	—	—	—	—	—	0.048	—
6	0.010	—	—	—	—	—	—	—	—	—	—	—	—
ME	$n = 37$	$n = 52^*$	$n = 43$	$n = 45$	$n = 20$	$n = 18$	$n = 2$	$n = 39$	$n = 38^*$	$n = 52$	$n = 34$	$n = 31$	$n = 30$
1	0.068	0.779	0.047	—	—	—	—	1.000	—	—	—	1.000	—
2	0.932	0.202	0.953	1.000	0.025	—	—	—	0.803	0.019	1.000	—	—
3	—	0.019	—	—	0.975	1.000	1.000	—	0.197	0.981	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	1.000
GPI	$n = 50$	$n = 53$	$n = 43$	$n = 46$	$n = 20$	$n = 11$	$n = 11$	$n = 39$	$n = 39^*$	$n = 52$	$n = 44$	$n = 31^*$	$n = 30^*$
1	0.030	0.009	0.070	—	0.025	—	—	0.115	—	—	—	0.016	—
2	0.010	—	—	—	—	—	—	—	—	—	—	0.065	—
3	0.870	0.934	0.826	0.891	0.950	0.864	0.864	0.885	0.077	—	—	0.565	0.550
4	0.010	—	—	—	—	—	—	—	0.013	—	—	0.355	0.417
5	0.060	0.057	0.105	0.109	0.025	0.136	0.136	—	0.833	1.000	0.977	—	0.033
6	0.010	—	—	—	—	—	—	—	0.077	—	0.023	—	—
7	0.010	—	—	—	—	—	—	—	—	—	—	—	—
AAT-2	$n = 50$	$n = 53$	$n = 44$	$n = 46$	$n = 20$	$n = 20$	$n = 11$	$n = 47^*$	$n = 39^*$	$n = 52$	$n = 44$	$n = 31$	$n = 30$
1	0.020	0.972	0.034	—	—	—	—	—	0.141	—	—	1.000	—
2	0.010	0.009	—	—	—	—	—	0.064	—	—	—	—	1.000
3	0.970	0.019	0.966	—	1.000	1.000	1.000	0.936	0.821	1.000	1.000	—	—
4	—	—	—	—	—	—	—	—	0.038	—	—	—	—
5	—	—	—	1.000	—	—	—	—	—	—	—	—	—

Table 2. Continued.

AAT-1	n = 50	n = 53	n = 44	n = 46	n = 20	n = 20	n = 11	n = 47*	n = 39*	n = 52	n = 44	n = 31	n = 30
1	0.050	0.019	0.023	—	—	—	—	0.085	—	0.019	0.045	—	—
2	0.950	0.981	0.977	—	1.000	0.950	1.000	0.915	0.846	0.981	0.932	1.000	—
3	—	—	—	0.989	—	0.050	—	—	0.154	—	0.023	—	1.000
4	—	—	—	0.011	—	—	—	—	—	—	—	—	—
LDH-2	n = 38	n = 35	n = 17	n = 22	n = 20	n = 20*	n = 5	n = 40	n = 26	n = 40	n = 22	n = 26	n = 26
1	0.961	—	—	—	—	—	—	—	—	—	—	—	—
2	0.039	1.000	1.000	0.841	0.275	0.075	1.000	1.000	0.019	0.175	1.000	0.962	1.000
3	—	—	—	—	0.725	0.875	—	—	0.942	0.712	—	—	—
4	—	—	—	0.159	—	0.050	—	—	0.038	0.112	—	0.038	—
LDH-1	n = 38	n = 26	n = 46*	n = 46	n = 20	n = 20	n = 5	n = 44	n = 36*	n = 49*	n = 32*	n = 24	n = 24
1	1.000	0.981	1.000	1.000	0.925	0.950	0.600	—	0.819	0.480	0.688	1.000	1.000
2	—	0.019	—	—	0.075	0.050	0.400	0.864	0.181	0.520	0.313	—	—
3	—	—	—	—	—	—	—	0.080	—	—	—	—	—
4	—	—	—	—	—	—	—	0.057	—	—	—	—	—
MDH-2	n = 42	n = 53	n = 44	n = 46	n = 12	n = 15	n = 2	n = 22*	n = 37	n = 51	n = 35	n = 31	n = 30
1	—	—	0.011	—	—	—	—	—	0.014	0.010	—	—	—
2	1.000	1.000	0.989	0.978	1.000	1.000	1.000	0.727	0.959	0.980	1.000	0.968	—
3	—	—	—	0.022	—	—	—	0.273	0.014	0.010	—	0.032	1.000
4	—	—	—	—	—	—	—	—	0.014	—	—	—	—
MDH-1	n = 50	n = 51	n = 44	n = 46	n = 20	n = 20	n = 3	n = 14*	n = 38	n = 52	n = 35	n = 31	n = 30
1	1.000	1.000	1.000	1.000	0.050	—	—	0.464	0.013	0.010	—	—	—
2	—	—	—	—	0.950	1.000	1.000	0.429	0.961	0.990	1.000	0.968	1.000
3	—	—	—	—	—	—	—	0.107	0.026	—	—	0.032	—
Mean sample size per locus	43.8 (2.1)	49.2 (3.1)	38.6 (2.8)	40.8 (3.3)	18.5 (1.0)	16.7 (1.3)	7.2 (1.2)	33.7 (4.3)	36.7 (1.1)	49.1 (1.7)	38.1 (2.2)	29.5 (0.8)	28.8 (0.6)
Mean number of alleles per locus	2.8 (0.6)	2.5 (0.4)	2.5 (0.5)	2.0 (0.3)	2.1 (0.4)	2.1 (0.4)	2.1 (0.4)	1.9 (0.2)	3.2 (0.4)	2.3 (0.3)	1.8 (0.3)	2.3 (0.4)	1.5 (0.2)
% P (95% criterion)	54.5	45.5	36.4	45.5	54.5	63.6	36.4	72.7	81.8	36.4	45.5	36.4	27.3
Mean heterozygosity	0.11 (0.04)	0.10 (0.03)	0.12 (0.04)	0.06 (0.02)	0.09 (0.03)	0.12 (0.05)	0.06 (0.03)	0.07 (0.03)	0.12 (0.05)	0.11 (0.05)	0.08 (0.04)	0.05 (0.02)	0.02 (0.01)

* Loci with heterozygote deficiencies, N number of individuals.

Table 3. Among-population variation (θ) for each locus and over all loci for *Gammarus* populations from the Chihuahuan Desert. *Nm:* number of migrants per generation.

Locus	θ
PGM	0.42
MPI	0.25
EST	0.18
ME	0.79
GPI	0.70
AAT-2	0.88
AAT-1	0.70
LDH-2	0.75
LDH-1	0.55
MDH-2	0.13
MDH-1	0.93
Over all loci	0.59

	Nm
Among all populations	0.1727
Among Toyah Creek populations (G, PL, SS, ES)	0.2774
Among Bitter Lake populations (BL1, BL2, BL3)	1.7825

combined into one single population (Y) for the remaining analyses due to these results, plus the lack of any rare or unique alleles among these populations, the most common allele being shared at 9 of 11 loci, and the hydrological connection among the sites.

Exact tests performed for each locus among the three Bitter Lake populations showed 9 of 11 loci (all but *MDH-1* and *MDH-2*) to be significantly heterogeneous (experiment-wise error rate $\alpha = 0.05$). The same test on the Toyah Creek populations (PL, G, SS, and ES) showed 7 of 11 loci significantly heterogeneous (*EST, AAT-1, AAT-2, LDH-2, ME, MPI, PGM*; experiment-wise error rate $\alpha = 0.05$).

High genetic variation was found among the *G. pecos* complex populations. Our estimate of the population sub-structure component of total genetic variation (θ) is well above Wright's (1978) suggested value of 0.25, which indicates high genetic differentiation among populations (Table 3). Estimated gene flow (number of migrants per generation; *Nm*) (Slatkin and Barton 1989) among all *G. pecos* complex populations was one migrant approximately every six generations ($Nm \approx 0.17$) (Table 3). This level of migra-

tion is much lower than $Nm \approx 1$, the level of gene flow required to prevent differentiation by genetic drift (Allendorf 1983). Similar results were found among Toyah Creek populations, whereas *Nm* among Bitter Lake populations was >1 (Table 3).

The *D* within the *G. pecos* species complex was 0.51 (± 0.26 SD); between *G. fasciatus* and the *G. pecos* species complex it was 0.73 (± 0.26); and between *E. ischnus* and the *G. pecos* complex it was 1.63 (± 0.58; Table 4). Of interest, *D* between *G. fasciatus* and population SS (0.40) was lower than the average *D* within the *G. pecos* species complex (Table 4). Moreover, *D* between SB and PL was higher than the genetic distances between *G. fasciatus* and all of the *G. pecos* complex populations. Genetic distances among Bitter Lake populations were below 0.10, while among Toyah Creek populations *D* ranged 0.07–0.46 (Table 4). Average genetic distances between all BL sites and Y are similar to average genetic distances among all sites in the Toyah Creek basin.

Cluster analysis showed the two populations from Lake Erie separated from the Chihuahuan Desert populations, with *G. fasciatus* more closely related to the *G. pecos* populations than to *E. ischnus* (Fig. 2). The analysis revealed four major population groups within the *G. pecos* complex: (1) Toyah Creek, (2) Sitting Bull, (3) Diamond Y Spring, and (4) Bitter Lake (Fig. 2). The bootstrap value between Bitter Lake populations and Diamond Y Spring supports the conclusion that the latter population is different from the Bitter Lake populations. However, the bootstrap values among the Toyah Creek populations and between Toyah Creek and remaining *G. pecos* populations are low, which suggests that further analysis may be required to understand relationships within this species complex.

The Mantel test between *D* and Cole's (1985) morphological similarities for the same populations used in this study showed a strong negative correlation between the two matrices ($r = -0.51$, $P = 0.025$; Fig. 3). These results suggest that genetically similar populations were also similar morphologically. The geographic distances among populations along water are presented in Table 4. The Mantel test for the correlation of geographic distance along-river and *D* demonstrated a significant correlation between the two matrices ($r = 0.64$, $P = 0.007$; Fig. 4). Similar results were found

Table 4. Pairwise Nei (1978) unbiased genetic distances (above diagonal) and pairwise geographic distances (km) along rivers (below diagonal). Population codes are given in Table 1.

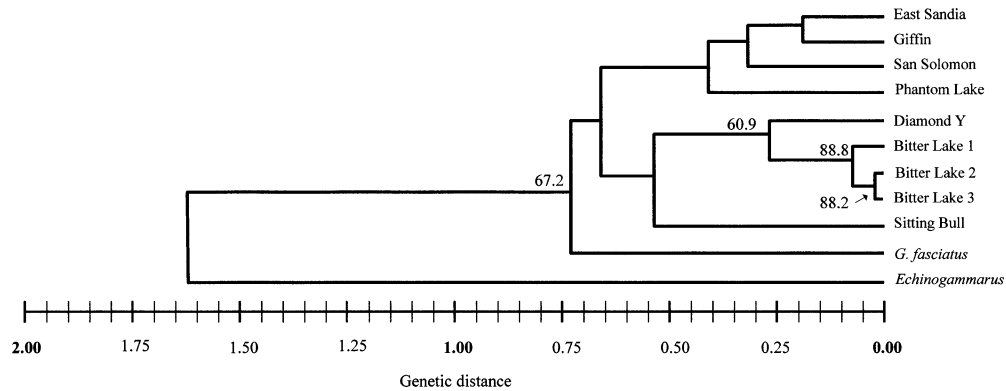| Populations	BL3	BL2	BL1	Y	PL	SS	G	ES	SB	*G. fasciatus*	*E. ischnus*
BL3		0.02	0.06	0.28	0.78	0.81	0.48	0.44	0.58	0.67	2.08
BL2	2		0.08	0.28	0.80	0.77	0.53	0.45	0.57	0.71	2.17
BL1	6	6		0.25	0.80	0.63	0.54	0.44	0.49	0.66	1.78
Y	739	739	734		0.84	0.57	0.50	0.40	0.49	0.72	1.86
PL	584	584	578	274		0.46	0.31	0.33	1.25	0.91	1.08
SS	587	586	581	350	7		0.31	0.21	0.61	0.40	1.50
G	577	577	572	267	7	1		0.07	0.79	0.55	1.40
ES	576	576	571	340	18	11	40		0.65	0.74	1.51
SB	276	276	271	598	443	445	436	435		1.01	1.65
G. fasciatus											1.58

Fig. 2. UPGMA phenogram based on Nei (1978) unbiased genetic distances among populations of the *G. pecos* complex, *G. fasciatus* from Lake Erie, and *E. ischnus* from Lake Erie. Numbers at nodes are the percentage of bootstrapped trees (out of 1,000 total trees) supporting the node. All other nodes were supported by <50% of the trees (range 10.7–47.2% of trees).

between *D* and overland geographic distance ($r = 0.68$, $P = 0.004$).

Discussion

Intrapopulation genetic variation in the G. pecos *complex*—Our results showed significant levels of genetic variation within populations of the *G. pecos* complex. Similar levels of intrapopulation variation were found in a survey of freshwater amphipods from eastern North America (Hogg et al. 2000). However, other isolated populations of amphipods exhibit much lower levels of variation within populations (Gooch and Glazier 1986; McPeek and Wellborn 1998). Our results do not support the current understanding of population genetics that small, well-differentiated, isolated populations should exhibit low intrapopulation genetic variation due primarily to evolutionary forces such as genetic drift, natural selection, and inbreeding (Hartl and Clark 1997). Hogg et al. (2000) suggested that the high levels of variation they observed in amphipod species was due to incomplete knowledge of amphipod taxonomy and the presence of cryptic species. Moreover, they suggested using Wright's *F* statistic F_{ST} (θ in our study) as an indication of the presence of cryptic species; large F_{ST} values indicate distinct subspecific

or specific status (Hogg et al. 2000). Our similar results suggest that cryptic species may be found within populations of the *G. pecos* complex.

The percentage of loci significantly different from HW expectations was extremely high compared with other amphipod species (for example, Hogg et al. 1998, 2000). Heterozygote deficiency seems to be a common feature of deep-sea invertebrates (Creasey et al. 1997). Three main processes are thought to have induced these deficiencies in marine crustaceans: natural selection, assortative mating, and the presence of cryptic species (Creasey et al. 1997). Usually, heterozygote deficiency caused by selection occurs only at specific loci, whereas it is found across all loci when it is due to inbreeding (Creasey et al. 1997). In the case of the *G. pecos* complex, the high number of loci significantly different from HW expectations and the resulting high inbreeding coefficients (*f*) suggest nonrandom mating as the main cause of heterozygote deficiency. Two populations, BL1 and SB, showed very high proportions of polymorphic loci, heterozygote deficiencies at over half of all loci sampled, and high values of inbreeding; together, these factors provide further evidence of the presence of cryptic species (Hogg et al. 2000). Indeed, in population BL1 there are two alleles at
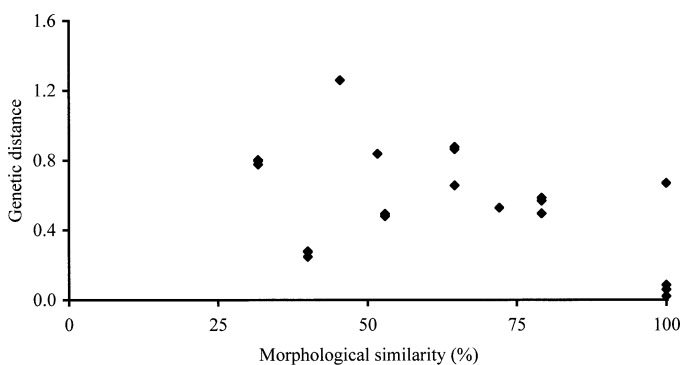
Fig. 3. Cole's (1985) morphological similarity vs. Nei (1978) unbiased genetic distance. Values are negatively correlated (Mantel test of arcsine-transformed morphological similarities, $r = -0.51$, $P = 0.025$).
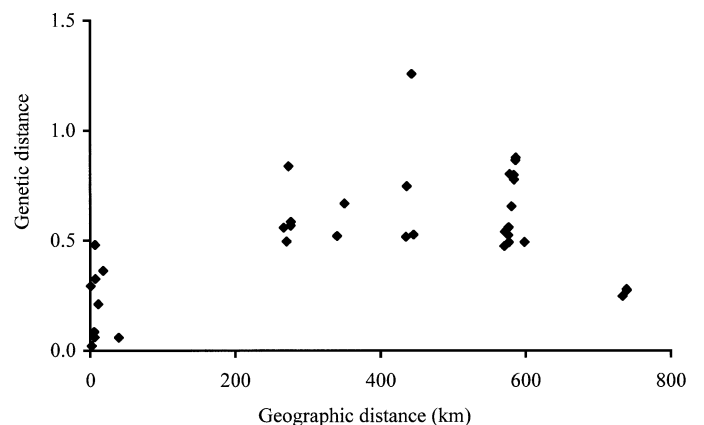
Fig. 4. Geographic distance along river vs. Nei (1978) unbiased genetic distances. Values are positively correlated (Mantel test of log-transformed data, $r = 0.64$, $P = 0.007$).

AAT-1 (Table 2), but no heterozygotes. The Sitting Bull population also has two alleles at *MPI, AAT-1, AAT-2,* and *MDH-2* (Table 2), but no heterozygotes at these loci. The high level of genetic diversity among the nine populations of the *G. pecos* complex ($\theta = 0.59$) further supports the hypothesis of the presence of cryptic species (Hogg et al. 2000). Both the BL1 and SB populations contain unique alleles. Field observations have shown differences in swimming behavior for some individuals from BL1 (B.K.L. pers. obs.); such behavioral differences are known to differentiate species within the amphipod genus *Hyallela* (Thomas et al. 1997). Preliminary morphological analysis indicates two forms of *Gammarus* at SB (B.K.L. pers. obs.). Both behavioral and morphological differences may be additional evidence of multiple species existing in sympatry. Further analyses of these populations are warranted.

In general, Cole's morphological analysis of the species complex (Cole 1985) is consistent with the results we report. The exception to this is his assigning the name *G. pecos* to both populations Y and SS. Although morphological similarity was 100% for these two populations, genetic distance between them is very large. The cluster analysis places SS with the other populations from the Toyah Creek basin, whereas Y is found in the other major branch within the *G. pecos* complex. Thus, SS is probably not *G. pecos,* but instead is part of the unresolved group of populations within the Toyah Creek basin (discussed later in the present article).

Genetic distance and geographic distance—The strong positive correlations between *D* and geographic distances suggest allopatric speciation of the *G. pecos* complex via isolation-by-distance (Vrijenhoek 1998). Similar results have been reported in desert amphipods of the genus *Hyallela* (Thomas et al. 1997). In more mobile branchiopod crustaceans like *Branchipodopsis wolfi,* even a distance <2 km was found to be a barrier to gene flow and led eventually to allopatric speciation (Brendonck et al. 2000). Low levels of gene flow have led to significant divergence of fairy shrimp (*Branchinecta coloradensis*) populations at distances of 5–10 km (Bohonak 1998). Thus, the limited ability to disperse and the lack of a specialized dispersal stage in *Gammarus* have likely played an important role in producing reproductive isolation. The low levels of gene flow estimated and the high level of genetic diversity among populations in the *G. pecos* complex are consistent with this hypothesis.

The limited effective dispersal capacity, together with short generation times, might have exposed these populations to divergent selection pressures because of habitat gradients (Witt and Hebert 2000). However, it is worth noting that the populations at the extremes of the geographic range, Bitter Lake and Diamond Y Spring, cluster together (Fig. 2). A similar result was seen by Hogg et al. (1998) when studying the population genetic structure of *Hyalella azteca* from the Laurentian Great Lakes basin. Their analysis of genetic distance revealed a cluster of similar populations ($D < 0.15$) occupying sites widely separated geographically (Hogg et al. 1998). The authors suggested that the individuals from those locations might have been a more widely distributed species, in contrast to genetically distinct populations from the other sites (Hogg et al. 1998). One potential

explanation for our results is that the Diamond Y and Bitter Lake habitats are most similar (N.L.A. pers. obs.). Alternatively, dispersal along the Pecos River may have allowed more recent gene flow between these locations, whereas the greater distance from the main stem of the Pecos to the Toyah Creek basin and the elevational climb required to reach Sitting Bull Spring may have limited migration to these latter areas. Either hypothesis might result in two lines of descent: Bitter Lake and Diamond Y Spring may have had more recent gene flow, whereas genetic distances among the other populations (Sitting Bull and Toyah Creek sites) might be explained by an isolation-by-distance model. The discovery of other populations at various distances from the Pecos River main stem (such as the Caroline Spring population) and use of other biochemical genetic markers may allow us to further test these hypotheses.

Relationship of Cole's (1985) populations of undetermined taxonomic affinity—To determine whether the populations of undetermined taxonomic affinity are genetically distinct from the described species, we examined genetic distances among pairs of populations and compared them with previously published standards for amphipod species boundaries. Thorpe (1982), in a survey of a variety of taxa, concluded that ~97% of the *I* values between species are <0.85 and that 98% of within-species values are >0.85. However, a survey of genetic studies on intra- and interspecific differentiation in amphipod populations suggested that a more conservative approach than Thorpe's was necessary (Stewart 1993). The latter study suggested that, when *I* ranges 0.45–0.85, concordant evidence of taxonomic distinction must be sought in fixed allelic differences for one or more loci and/or morphological differences. Genetic identities among our study populations indicate that the three Bitter Lake populations all belong to the same species ($I > 0.85$). This result is consistent with Cole's (1985) morphological analyses. Populations ES and G should be considered the same species ($I > 0.85$). On the other hand, populations PL and Y should be considered different species ($I < 0.45$), as well as *G. fasciatus* and population PL ($I < 0.45$). For the other values in the 0.45–0.85 range, fixed allelic differences were examined. Three distinct groups were found: Bitter Lake populations, Diamond Y Spring, and Sitting Bull. Still unclear is the taxonomic relationship of populations ES/G, PL (*G. hyalleloides*), and SS. Again, the results confirm Cole's (1985) finding that Bitter Lake populations (*G. desperatus*), the Diamond Y Spring population (*G. pecos*), and the Sitting Bull population (described by Cole (1985) as "morphologically distinct") are separate species. Our results disagree with Cole's determination that *G. pecos* is found in San Solomon Spring. The significant correlation between *D* and Cole's (1985) morphological similarities suggests that, in general, the genetic data are in accord with the morphological data.

Our study revealed significant intrapopulation genetic variation and considerable genetic diversity among populations of the *G. pecos* complex. High levels of polymorphism accompanied by heterozygote deficiencies with respect to HW expectations, extremely high values of the inbreeding coefficient, *f*, and distinct genotypes at single loci suggest the

presence of cryptic species in at least two of the nine studied populations (BL1 and SB). Nei (1978) genetic identities, fixed allelic differences, and unique alleles revealed four groups within the *G. pecos* complex: Bitter Lake, Diamond Y Spring, Sitting Bull, and the Toyah Creek basin. From these results, we suggest the presence of at least four distinct species: *G. desperatus* (Bitter Lake), *G. pecos* (Diamond Y Spring), *G. hyalleloides* (Phantom Lake), plus an undescribed species at Sitting Bull. Further analyses are necessary to clarify the taxonomic relationship of the other populations in the Toyah Creek basin and to elucidate the presence of cryptic species/subspecies in populations BL1 and SB. Thus, the *G. pecos* complex likely consists of at least four unique taxonomic entities, each of which is relegated to an isolated spring system. In addition, several sites may contain cryptic species. Further morphological and genetic analyses, perhaps combined with breeding experiments, are necessary to delineate the taxonomic affinities of the populations that are still unresolved.

Regional patterns within the Chihuahuan Desert—The UPGMA analysis showed that all *G. pecos* complex populations were more similar to each other than to the two outgroups. The two populations from Lake Erie were well-separated from the Chihuahuan Desert populations, and *G. fasciatus* was more closely related to the *G. pecos* populations than to *E. ischnus* (Fig. 2). Thus, Chihuahuan Desert *Gammarus* species appear to form a distinct group.

The phenogram is similar to results reported by Echelle et al. (1989) in their study on genetic diversity in populations of *Gambusia nobilis,* an endangered fish that is endemic in Chihuahuan Desert springs. The fish and amphipods not only share the same habitat, but our collection sites are in close proximity. Echelle et al. (1989) found the same four major population groups that we found: the Toyah Creek populations, Diamond Y Spring population, Bitter Lake populations, and the Blue Spring population (geographically comparable to our Sitting Bull Spring population). Heterogeneity among populations was highest in the Toyah Creek basin for both gambusia and amphipods. The cluster analysis performed on *G. nobilis* showed results similar to ours, with Bitter Lake and Diamond Y populations clustering together even though they are the furthest apart geographically. A Mantel test performed on genetic distances between populations within the *G. pecos* complex and the corresponding *G. nobilis* populations showed a strong correlation (r = 0.81; Fig. 5). However, genetic distances between pairs of amphipod populations were all at least seven times greater (and 9 of 10 comparisons were more than an order of magnitude greater) than those between corresponding *G. nobilis* populations (range 0.002–0.101). These differences are likely due to the greater dispersal ability of fishes and are reflected in the fact that only a single species of *Gambusia* has been recognized from these sites, whereas the amphipods comprise a species complex.

Several endemic species of inland pupfishes (*Cyprinodon* spp.) are also found in this region. Allopatric species within this group are found in the vicinity of BLNWR (BL), at San Solomon Spring (SS), and in Diamond Y Draw (Y). Genetic distances among these populations (calculated from results
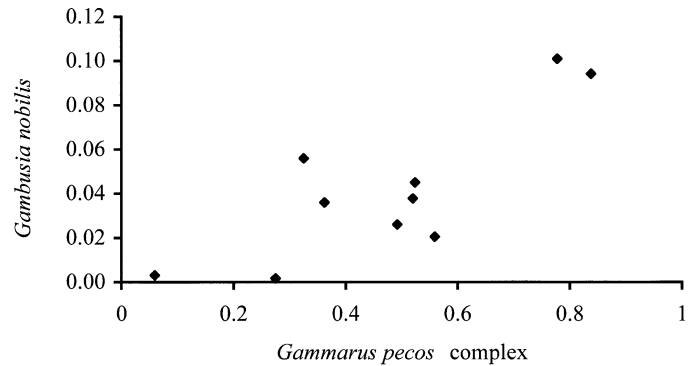

Fig. 5. Nei (1978) unbiased genetic distances among pairs of populations of the *G. pecos* species complex and pairs of populations of *G. nobilis* from the same locations (r = 0.81, P = 0.041; Echelle et al. 1989). Echelle et al. (1989) sampled several sites at Diamond Y; we used the means of these for comparison with our site Y.

in Echelle and Echelle 1992) are greater than comparable populations of *G. nobilis* but less than that of the comparable amphipod populations (pupfish distances 0.13–0.62). For all three of these taxa, populations near BL were more similar to those from Y than to those in the Toyah Creek basin, even though the latter sites are ~150 river-km closer to the BL sites. For all three taxonomic groups, greater heterogeneity was found within the Toyah Creek basin than in other locations (Echelle et al. 1989). The correlation of these geographic patterns across taxa suggests that similar evolutionary forces may be acting on unrelated groups of organisms. The degree of isolation (and, thus, the genetic distances) among populations within each group may reflect either dispersal ability of the group or, alternatively, the amount of time since each of these groups colonized the northern Chihuahuan Desert.

A similar coincidence in the geographic patterns of the genetic structure of codistributed species has been noted in the southeastern United States (Avise 1993). In this region, the distinct east versus west genetic pattern present among populations of several taxa has been explained by the influence of historical drainage isolations and connections that characterized the region from the Pliocene to the Pleistocene (Avise 1993). In the freshwater realm, biogeographic factors such as isolation and connection of drainages affect the whole biotic community. Therefore, species sharing similar ecosystem requirements should become genetically structured in a geographically similar way, although the magnitude of the populations' genetic separation may vary depending on the dispersal characteristics of the species and also on the genetic variability present in the species at the moment of the biogeographic changes (Avise 1993). Although our results show that patterns of amphipod, gambusia, and pupfish genetic distances are similar, distance values were highest for amphipods, intermediate for pupfish, and lowest for *Gambusia*. Presumably, this is due to the greater dispersal ability of the latter and is reflected in the taxonomy of these groups (multiple amphipod and pupfish species vs. several populations within a single described species of *Gambusia*). Aquatic snails of the family Hydrobiidae from

this region (which are presumably even less vagile than amphipods) showed very high levels of variation among these same sites, with multiple species and genera present in the Pecos basin (Hershler et al. 1999).

From a conservation perspective, the differences within the *G. pecos* complex suggest that each population should be considered to be unique and that strategies to preserve its status should be developed. If one of these isolated populations is extirpated, the genetic loss may be irretrievable. Moreover, the strong correlation between the *G. pecos* complex and other aquatic taxa from the same sites suggests that similar evolutionary forces may be acting on unrelated groups of organisms inhabiting these spring systems. Further analyses of other taxa might clarify the extent of this phenomenon. Given the uniqueness of the Chihuahan Desert region and its extremely fragile aquatic habitats, it is imperative that further destruction of habitat be minimized in order to ensure preservation of these unique faunal assemblages.

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