GENETIC VARIATION WITHIN AND AMONG POPULATIONS OF FAIRY SHRIMP, *STREPTOCEPHALUS TEXANUS*, FROM SOUTHEASTERN UTAH

Richard Halliburton* and Tim B. Graham

Department of Biology, Western Connecticut State University, Danbury, CT 06810, USA (RH)
Department of Geography, University of Utah, 332 South 1400 East, Room 217, Salt Lake City, UT 84112 (TBG)
**Correspondent: halliburtonr@gmail.com*

Abstract—To estimate levels of gene flow over short distances (less than a few kilometers) in the fairy shrimp, *Streptocephalus texanus*, mitochondrial DNA sequences (part of the cytochrome oxidase locus) were examined in 98 individuals from 13 populations near Moab, Utah. Populations were arranged linearly along two transects on slickrock ridges, and one isolated population about 2–3 km from the others. We found six haplotypes, differing from one another by 1 to 23 nucleotides. Nucleotide diversity among the nine populations from the MiVida ridge was 0.0019 with no isolation by distance. The three populations from the Sand Flat ridge were fixed for the same haplotype, not found in other populations. The isolated population (loop B pool) contained two haplotypes, one of which was very different from others in the study. The general pattern was little differentiation among populations separated by less than about 1 km, with more differentiation at greater distances. This is consistent with passive dispersal of cysts by wind over short distances, and rare long-distance dispersal.

Resumen—Para estimar los niveles de flujo de genes a través de distancias cortas (menos de unos pocos kilómetros) en el camarón hada, *Streptocephalus texanus*, se examinaron secuencias de ADN mitocondrial (parte del locus citocromo oxidasa) en 98 individuos de 13 poblaciones cerca de Moab, Utah, USA. Las poblaciones se organizaron linealmente a lo largo de dos transectos en crestas de rocas pulidas, y una población aislada de unos 2–3 km de los otros. Encontramos 6 haplotipos que difirieron uno del otro de 1 a 23 nucleótidos. La diversidad de nucleótidos entre las nueve poblaciones de la cresta MiVida fue de 0.0019 sin aislamiento por distancia. Las tres poblaciones de la cresta de Sand Flat fueron fijadas para el mismo haplotipo, no encontrado en otras poblaciones. La población aislada LBP contuvo 2 haplotipos, uno de los cuales fue muy diferente de otros del estudio. El patrón general era de poca diferencia entre las poblaciones separadas por menos de 1 km aproximadamente, con una mayor diferencia a mayores distancias. Esto es consistente con la dispersión pasiva de quistes por el viento a través de distancias cortas, y rara dispersión a larga distancia.

Slickrock areas of the Colorado Plateau in the southwestern United States are littered with depressions called desert potholes. These potholes contain wind-blown sediment and are dry during much of the year, collecting water during seasonal rains. Sizes generally range from less than a meter to several meters across, and depth is usually less than a meter. They hold water from a few days to several weeks, depending on size. These potholes are inhabited by numerous organisms adapted to this alternating wet–dry cycle, including fairy shrimp (Anostraca) *Branchinecta packardi* and *Streptocephalus texanus*, clam shrimp *Leptestheria compleximanus* and *Eulimnadia inflecta*, and tadpole shrimp (Notostraca) *Triops longicaudatus* (Graham, http://geochange.er.usgs.gov/sw/impacts/biology/vernal). These crustaceans survive dry periods as dormant cysts in the sediment of potholes. Dispersal of all branchiopods is only by passive means, as other stages cannot survive out of water.

This unusual life cycle, with small local populations, overlapping generations, and discontinuous distribution, raises interesting questions about levels of genetic differentiation and short- and long-distance gene flow via animal and wind dispersal among different potholes. Levels of divergence depend, at least in part, on levels of gene flow. If dispersal is passive and gene flow is positively correlated with dispersal distance, this should result in greater differentiation among distant populations than among nearby populations, perhaps with an isolation-by-distance effect.

Several studies have addressed this issue in anostracans. The earliest reports were based on allozymes (e.g., Davies et al., 1997; Bohonak, 1998; Fugate, 1998;

Brendonck et al., 2000). In general, these studies found low differentiation (as estimated by F_{ST}) among populations separated by about 10 km or less, and much greater differentiation among populations separated by tens to hundreds of kilometers, with no clear geographic pattern.

Studies based on mitochondrial deoxyribonucleic acid (mtDNA) sequence variation (typically the cytochrome oxidase subunit I locus) are generally consistent with the allozyme studies. High levels of differentiation among populations have been seen in several species of fairy shrimp (e.g. Fugate, 1998; Brendonck et al., 2000; Ketmaier, et al., 2005; McCafferty et al., 2010; Aguilar, 2011; Vanschoenwinkel et al., 2011; Pinceel et al., 2013; Zarattini et al., 2013). Most studies have concentrated on populations separated by tens to hundreds of kilometers. Limited data exist on populations separated by a few kilometers or less, but several studies show high differentiation over short distances (e.g. Zofkova and Timms, 2009; McCafferty et al. 2010; Vanschoenwinkel et al., 2011; Zarattini et al., 2013). For example, Zarattini et al. (2013) found significant pairwise F_{ST} values (49% of 55 comparisons) among populations of Italian *Chirocephalus ruffoi* separated by a few hundred meters. McCafferty et al. (2010) found that, of three pairs separated by less than 1 km, two showed high levels of divergence.

These results are generally consistent with the idea that limited passive dispersal severely restricts gene flow among distant populations. What is the critical distance beyond which gene flow is so limited that populations can diverge substantially, due to either random or selective factors? This raises questions about mechanisms of passive dispersal. Possibilities include wind, transport of propagules on the feet or in the gut of birds or other animals, or spillover between nearby pools during storms (e.g., Bohonak and Whiteman, 1999; Hulsmans et al., 2007; Graham and Wirth, 2008; Green et al., 2008; Vanschoenwinkel et al., 2008*a*, 2009; Brochet et al., 2010). The relative importance of these mechanisms is poorly understood in aquatic invertebrates, and certainly varies among species in different environments (e.g., Bohonak, and Jenkins, 2003). This is important, since different mechanisms can have very different effects on dispersal distance. For example, dispersal of propagules on the feet or feathers of birds might have a much greater mean dispersal distance than dispersal by wind or by amphibians moving from one pool to another. Graham and Wirth (2008) argued that wind is the most important dispersal mechanism of branchiopod cysts on the Colorado Plateau.

The purpose of this study was to examine the relationships between dispersal distance, gene flow, and genetic differentiation among nearby populations of *Streptocephalus texanus*. We surveyed mtDNA variation in populations from potholes in the region near Moab, Utah, with concentration on populations separated by <1 km to approximately 3 km. If gene flow is limited by dispersal of cysts by wind, as Graham and Wirth (2008) argued, we expected to see a fairly sharp increase in divergence among populations beyond some relatively short distance.

Materials and Methods—*The Organism*—Fairy shrimp (Branchiopoda: Anostraca) are an ancient and taxonomically diverse group, consisting of about 500 species worldwide (Brendonck et al., 2008). In North America there are 11 genera with 64 species (Brendonck et al., 2008).

The genus *Streptocephalus* consists of about 60 species, six in North America (Belk and Brtek, 1995, 1997; Daniels et al., 2004). *Streptocephalus texanus* inhabits the desert potholes throughout the southwestern United States and extending eastward to the Great Plains (Eriksen and Belk, 1999). Adults can exceed 25 mm in length, and hatch from dormant cysts during warm seasonal rains. They mature quickly (within 7 days at warm temperatures), live for only a few days, and mate frequently. Females lay eggs every day or two until the pothole dries out or water temperature gets too cold. Eggs develop into cysts that remain dormant in the dry sediment until the next rain. Some cysts remain dormant through several wet–dry cycles before they hatch (Hildrew, 1985; Brendonck, 1996; Simovich and Hathaway, 1997). Graham and Wirth (2008) hypothesized that in this region cysts are dispersed primarily by wind.

Study Area and Collections—Adults were collected from 13 potholes (populations) near Moab, Utah (Table 1). Potholes were arranged linearly along two slickrock ridges ("fins"). Nine potholes were sampled on the MiVida (MV) ridge and three from the Sand Flat (SF) ridge. The ridges run roughly SE to NW. The most distant MV populations sampled were approximately 926 m apart, and the most distant SF populations, approximately 119 m (Table 2). The shortest distances between sampled potholes were 30 m and 63 m for MV and SF potholes respectively (Table 2). Distances between MV and SF populations were 1–2 km. In addition, one population, designated LBP (loop B pool), was sampled from near a campground on SF ridge. LBP is about 1,616 m from SF5, the nearest other pool sampled. It is an average of 3,019 m from the MV populations. Half the pairwise distances are less than 1 km. The maximum distance between two sampled populations is 3.7 km (Table 2).

Laboratory Procedures—All samples were stored in ethanol until they were processed. Genomic DNA was extracted using the Qiagen DNeasy blood and tissue kit (Qiagen, Inc., Germantown, Maryland). Extracted DNA was then purified using Sure-Clean Plus with the colorless coprecipitant (Bioline, Inc., Taunton, Massachusetts) Purified DNA was resuspended in 100 µL of Tris-EDTA, diluted to 10 ng/µL, and stored at −20°C until further processing.

Part of the cytochrome oxidase, subunit I (COI) mitochondrial gene was amplified by polymerase chain reaction using the primers HCO and LCO (Folmer et al., 1994). Reactions were conducted in 25-µL volumes as follows: 4.0 µL of template DNA (~1.0 ng/µL), 0.1 µL of Taq DNA polymerase (5 U/µL; Qiagen, Inc.), 2.5 µL of buffer (10×), 2.0 µL of bovine serum albumin (1 mg/mL), 0.5 µL of deoxynucleotide triphosphate mix (10 mM each), 2.0 µL of each primer (10 µM). The amplification reaction was performed with Bio-Rad iCycler (Bio-Rad Laboratories, Inc., Richmond, California) using the following program: template denaturation at 96°C for 6 min; 35 cycles of denaturation at 96°C for 30 s, primer annealing at 50°C for 45

TABLE 1—Locations, sample sizes (N), and summary statistics for 13 populations of fairy shrimp, *Streptocephalus texanus*. UTM coordinates refer to universal transverse Mercator, zone 12S, datum NAD27; s.d. is standard deviation.

Population	UTM East	UTM North	N	Haplotypes	Polymorphic sites	Haplotype diversity	s.d.	Nucleotide diversity	s.d.
MV02	626535	4272319	2	h1	0	0	—	0	—
MV03	626461	4272335	5	h1	0	0	—	0	—
MV07b	626415	4272364	9	h1, h2		0.5	0.128	0.000761	0.002
MV11	626390	4272379	7	h1	0	0	—	0	—
MV16a	626355	4272404	4	h1, h2	1	0.667	0.204	0.001015	0.00031
MV16b	626357	4272431	9	h1, h2	1	0.5	0.128	0.000761	0.0002
MV20	626307	4272453	9	h2, h3	2	0.222	0.166	0.000676	0.00051
MV30	626119	4272573	10	h1, h2, h4	23	0.378	0.181	0.007002	0.00514
MV42	626754	4272816	10	h1, h5	1	0.533	0.095	0.000812	0.00014
SF05	627469	4271636	4	h6	0	0	—	0	—
SF14b	627435	4271689	10	h6	0	0	—	0	—
SF15	627370	4271702	10	h6	0	0	—	0	—
LBP	628991	4270889	9	h1, h4	22	0.5	0.128	0.016743	0.0043
Regions:									
MV			65	h1-h5	25	0.55	0.047	0.00191	0.00099
SF			24	h6	0	0	—	0	—
LBP			9	h1,h4	22	0.5	0.128	0.01674	0.0043
Overall			98	h1-h6	25	0.691	0.029	0.00431	0.00115

s, and primer extension at 75°C for 2 min; and a final extension step of 72°C for 8 min. Successful amplification was confirmed by agarose gel electrophoresis in 2% Tris–acetic acid–EDTA gels. Amplification was considered successful if a single band of approximately 700 base pairs appeared on the gel.

Samples that amplified successfully were sent to the DNA Analysis Facility at Yale University and sequenced using their full-service sequencing that included Exo-SAP purification. Samples were sequenced in both directions. Sequences were edited and ambiguities resolved using FinchTV version 1.5 (Geospiza Inc., Seattle, Washington) and MEGA, version 6 (Tamura et al., 2013).

Data Analysis—Sequences were aligned and trimmed with ClustalW (Higgins et al., 1994), as implemented by MEGA6. General descriptive statistics and estimates of haplotype diversity and nucleotide diversity (π) were obtained using DnaSP version 5.10 (Librado and Rozas, 2009), Arlequin 3.5 (Excoffier and Lischer, 2010), and MEGA6.

Analysis of molecular variance (AMOVA), as implemented in Arlequin 3.5, was used to quantify variation due to differences among populations and among regions (MV vs. SF vs. LBP). Estimates of G_{ST} and N_{ST} for each region and overall were calculated with DnaSP and MEGA6. Mean within-group and between-group p-distances (proportion of different nucleotides between two sequences) were calculated with MEGA6.

Mantel tests, to assess the relationship between genetic distance and geographic distance, were calculated using PASSAGE, version 2 (Rosenberg and Anderson, 2011).

A haplotype network was constructed using TCS (Clement et al., 2000) based on the statistical parsimony methods of Templeton et al. (1992).

TABLE 2—Distances (m) between populations of *Streptocephalus texanus* sampled from potholes near Moab, Utah (MV = MiVida ridge, SF = Sand Flat ridge, LBP = loop B pool)

Pothole	MV02	MV03	MV7b	MV11	MV16a	MV16b	MV20	MV30	MV42	SF05	SF14b	SF15
MV02												
MV03	75.8											
MV7b	128.1	54.1										
MV11	157.7	84.1	30.0									
MV16a	217.7	143.9	89.9	60.0								
MV16b	210.5	141.4	88.8	60.9	34.5							
MV20	264.9	194.3	140.5	110.9	56.5	54.8						
MV30	487.9	417.0	362.9	333.0	274.4	277.5	223.1					
MV42	925.9	855.1	801.0	771.1	712.1	715.4	661.3	438.2				
SF05	1,157.0	1,226.7	1,280.9	1,310.8	1,369.9	1,367.0	1,420.8	1,643.7	2,081.8			
SF14b	1,097.9	1,168.2	1,222.3	1,252.3	1,311.6	1,308.1	1,362.1	1,585.1	2,023.3	63.5		
SF15	1,038.0	1,107.6	1,161.7	1,191.6	1,250.7	1,247.9	1,301.7	1,524.6	1,962.7	119.2	66.0	
LBP	2,746.1	2,819.6	2,873.5	2,903.4	2,963.4	2,955.7	3,010.4	3,232.6	3,668.4	1,616.2	1,667.3	1,732.3

TABLE 3—Cytochrome oxidase subunit I (COI) haplotypes of *Streptocephalus texanus*, frequencies, and populations in which they occurred. Numbers in parentheses are GenBank accession numbers.

	Haplotypes					
Population	h1 (KT583296)	h2 (KT583297)	h3 (KT583298)	h4 (KT583299)	h5 (KT583300)	h6 (KT583301)
MV02	2					
MV03	5					
MV07	3	6				
MV11	7					
MV16a	2	2				
MV16b	6	3				
MV20		8	1			
MV30	8	1		1		
MV42	6				4	
SF05						4
SF14b						10
SF15						10
LBP	6			3		
Regions:						
MV	39	20	1	1	4	
SF						24
LBP	6			3		
Total	45	20	1	4	4	24

RESULTS—*Overall Variation*—The aligned and trimmed sequences contained 657 nucleotide positions, with no gaps. There were 25 variable sites. All nucleotide substitutions were silent (no change in amino acid sequence) except one (site 623 in haplotype h3; Table 3).

Six different haplotypes were identified (Table 3). The number of differences between haplotypes ranged from 1 to 23. Haplotype h4, found in MV30 and LBP, was the most distinct, differing from the others at 20–23 positions. Haplotype h1 was the most frequent, found in 45 individuals and 10 populations, whereas h3 was the least frequent, found in only one individual (Fig. 1).

Variation within Populations—Of the 13 populations, six were monomorphic (Table 1). All three SF populations were monomorphic for the same haplotype, h6, which was not found in MV or LBP. Only MV30 had more than two haplotypes (three). Nucleotide diversities within variable populations ranged from 0.00068 in MV20 to 0.01674 in LBP (Table 1). Overall nucleotide diversity was 0.0043.

Regional Variation and Differentiation—We partitioned the populations into three geographic regions, MV, SF, and LBP, on the basis of topography and distance. LBP, with only a single population (nine individuals), was the most variable, with $\pi = 0.017$ (Table 1). MV (nine populations, 65 individuals) was much less variable, with $\pi = 0.0019$. Region SF (three populations, 24 individuals) was invariant.

Differentiation among regions was estimated by G_{ST} and N_{ST}. G_{ST} is based on haplotype diversity, and considers only the frequencies of haplotypes. N_{ST} is based on nucleotide diversity, and considers both the frequencies and sequences of haplotypes. These statistics can be interpreted as the proportion of total gene diversity that is due to differences in haplotype frequencies among regions (G_{ST}) and the proportion of total nucleotide diversity that is due to differences in nucleotide sequence among regions (N_{ST}). The estimates were qualitatively similar: MV and LBP were most similar ($G_{ST} = 0.044$, $N_{ST} = 0.218$), primarily due to the shared haplotype h4, the most distinct haplotype. SF was most different, sharing no haplotypes with either MV or LBP ($G_{ST} = 0.417$ or 0.686; $N_{ST} = 0.764$ or 0.309).

Analysis of molecular variance partitions the nucleotide variation into differences among regions, differences among populations within regions, and differences within populations (Table 4). About 54% of all variation is due to differences among regions, whereas only about 1% is due to differences among populations within regions; i.e., differences among regions account for much of the overall variation, but populations within a region are very similar.

Given significant divergence among all populations, we examined whether there was a relationship between geographic distance and genetic distance. A plot of geographic distance vs. mean *p*-distance for all pairs of populations shows clear isolation by distance (Fig. 2, regression line). The Mantel test for all three regions combined is highly significant (0.864; $P < 0.001$). However, that is heavily influenced by the comparatively great geographic distances of LBP from the other populations (the points in the topmost part of Fig. 2). Eliminating LBP from the analysis reduces the Mantel correlation from 0.864 to 0.714, still highly significant ($P < 0.001$). Considering only the MV populations (the

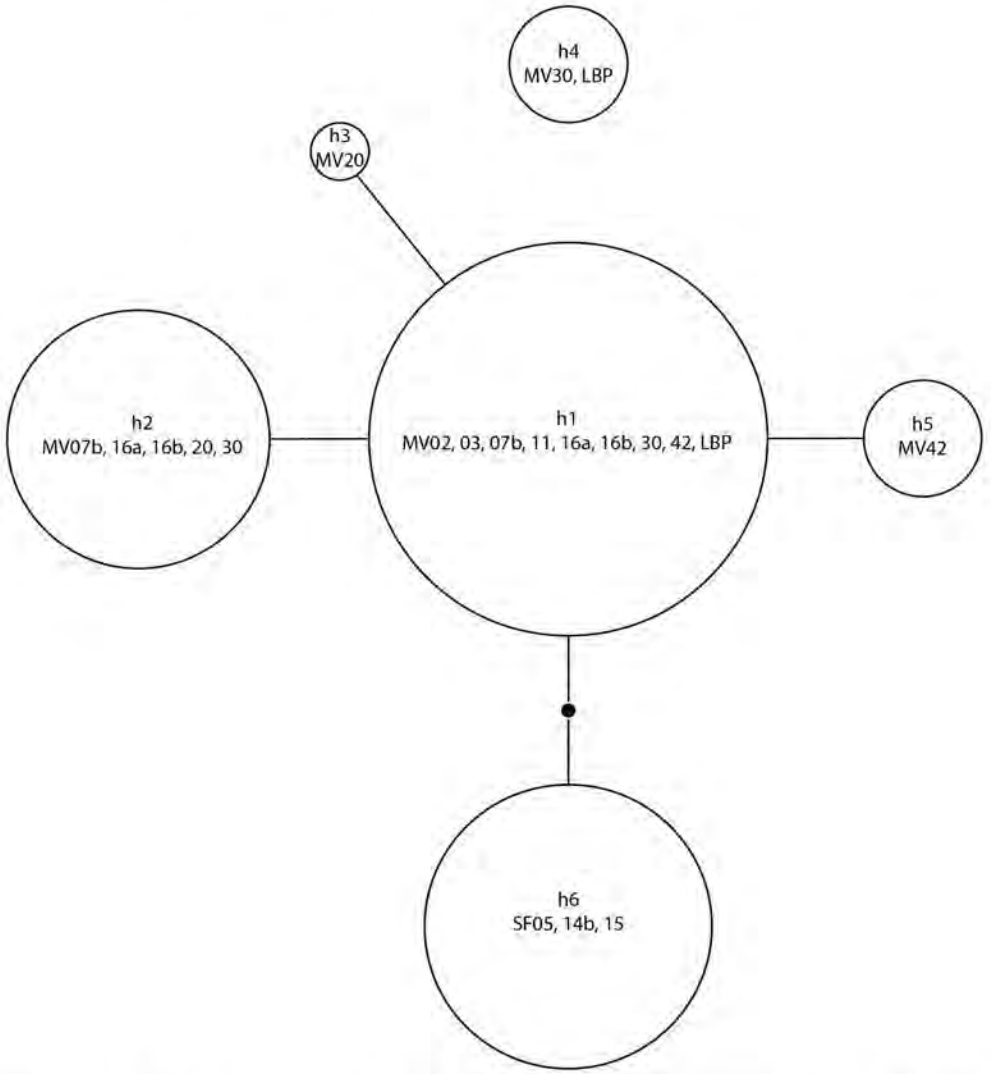

FIG. 1—Haplotype network showing relationships between cytochrome oxidase subunit I (COI) haplotypes in *Streptocephalus texanus*. Haplotype h4 could not be connected to the network with 95% probability. Areas of circles are approximately proportional to haplotype frequency. The small dot between h1 and h6 represents a hypothetical intermediate haplotype not found.

open circles in Fig. 2), the relationship is not significant ($P \approx 0.58$).

DISCUSSION—We found fewer haplotypes (six haplotypes in 98 sequences) than most other studies of fairy shrimp variation. For example, Aguilar (2011) found 39 haplotypes in 107 sequences of *Branchiodopsis Branchinecta lynchi*; Bohonak (in litt.) found 50 haplotypes in 316 sequences of *B. sandiegoensis*; Zofkova and Timms (2009) found 39 haplotypes in 61 sequences of *Branchinella longirostris*. The lower number of haplotypes in our study is probably because the geographic scale of our study was much smaller than in other studies. At similar scales, results are similar. McCafferty et al. (2010) in a study most similar in scale to ours, but larger, found 13 haplotypes in 122 sequences of *Eubranchipus vernalis*. Vanschoenwinkel

TABLE 4—Analysis of molecular variance for 13 populations of *Streptocephalus texanus* in three regions.

Source of variation	df	Sum of squares	Percent of variation	Probability
Among regions	2	53.23	54.3	0.001
Among populations, within regions	10	10.37	1.2	0.032
Within populations	85	73.88	44.5	<0.001
Total	97	137.48		

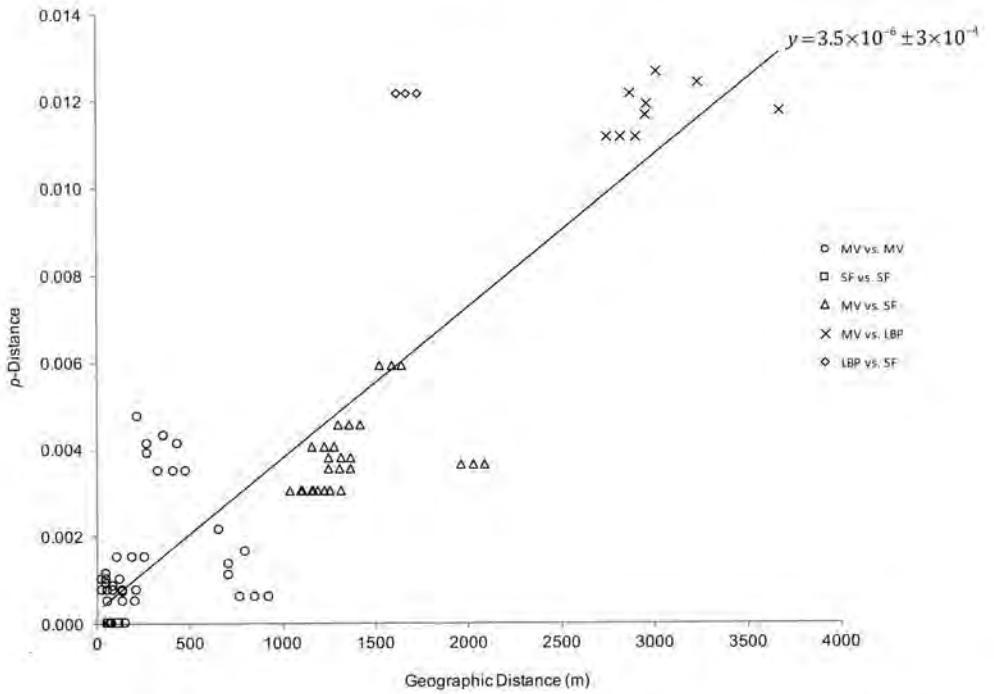

FIG. 2—Geographic distance vs. *p*-distance within and among regions for *Streptocephalus texanus*. The regression line is for all regions combined.

et al. (2011) found seven haplotypes of *Branchiodopsis wolfi* in a cluster of eight pools within about 100 m of one another.

Nucleotide diversity within variable populations ranged from 0.000676 to 0.0167, with six invariant populations, and an overall average of 0.00431. This is similar to other studies of fairy shrimp. For example, McCafferty et al. (2010) in a study of 22 Massachusetts populations of *Eubranchipus vernalis* found nucleotide diversity within 14 variable populations ranging from 0.00133 to 0.0101 with an overall average of 0.00213. Zarattini et al. (2013) found nucleotide diversities between 0.0009 and 0.0498 in 10 variable populations of *C. ruffoi* in Italy.

With the exception of h4, haplotypes were very similar, differing from one another at only one or two positions (Fig. 1). Haplotype h4 was quite different from the others, differing at 20–23 positions. Surprisingly, it was found in two distant populations, LBP and MV30, about 3.2 km apart. Haplotype h1, the most common haplotype, was also found in both LBP and MV30. These results suggest some gene flow between LBP and MV30.

In addition, Daniels et al. (2004) sequenced part of the COI region of a single *S. texanus* individual from New Mexico. Their sequence overlapped with ours for 598 nucleotides. Their haplotype differed from our h4, at only 11 positions. In other words, h4 was more closely related to the New Mexico haplotype, about 500 km distant, than it was to other haplotypes within the same population (MV30 or LBP).

We considered LBP a separate region, but in fact it is a single isolated pool, about 1.6 km from the nearest other pool sampled. Its nucleotide diversity was an order of magnitude higher than any other pool, due mostly to the presence of haplotype h4. LBP was the largest pool sampled and the most accessible, only a few meters from a road. Its large size might support a larger, more stable population, less likely to lose genetic variation by genetic drift or other stochastic processes. It is also possible that humans have transferred cysts from other pools. Hikers commonly walk through the sediment of dry pools, possibly picking up cysts on their boots. Also, vehicles are occasionally driven through this pothole and some could be carrying mud and cysts from other, perhaps distant, pools. This too might have contributed to the high diversity of LBP.

The distinctiveness and lack of diversity among SF populations is somewhat surprising. There is nothing obviously different about the SF and MV fins or the potholes on them. The lack of variation in SF could simply be sampling artifact, as we sampled fewer populations and fewer individuals than for MV. However, MV populations with similar sample sizes and separated by similar distances showed variation both within and among populations. Alternatively, it might be due to the "monopolization hypothesis" (De Meester et al., 2002) in which founder effects followed by rapid local adaptation and population growth to near carrying capacity combine to limit the success of subsequent immigrants. For example, the SF populations might be affected by runoff

from nearby uplands, which might affect water quality and sediment composition. More extensive sampling and fieldwork is needed to clarify this issue.

We found significant differences among different regions, but little differentiation among populations within a region (Table 4). Isolation by distance occurred among regions (Fig. 2), but not within MV, the only region with more than one variable population. This suggests moderate gene flow over distances less than 1 km (within MV and within SF) but more restricted gene flow at distances greater than 1 km; MV and SF populations are about 1–2 km apart and share no haplotypes. It appears that, at least for *S. texanus* in this environment, a distance of approximately 1 km is enough to severely restrict gene flow. A glaring exception is MV30 and LBP, about 3.2 km apart but sharing haplotypes h1 and h4. This indicates that some gene flow occurs over greater distances.

Other studies have inferred restricted gene flow on the scale of a few kilometers. Brendonck et al. (2000) studied four allozyme loci in 17 populations, divided into three metapopulations, of *Branchipodopsis wolfi* in southern Africa. More than 90% of variation among all populations was explained by variation among metapopulations (analogous to our regions), although they found that genetic differentiation did not correlate with either geographic distance or environmental variables (Brendonck et al., 2000). They suggested that 2 km or less may substantially reduce gene flow in *B. wolfi*. In a related study, Hulsmans et al. (2007) concluded that only 50 m might be an effective barrier to gene flow in this species.

Bohonak (in litt.) examined 75 populations (316 individuals) of *Branchinecta sandiegonensis* in southern California. The 50 mtDNA haplotypes clustered into two deeply separated clades that were allopatric, but not geographically continuous, with no relationship to known geologic features. He found high levels of divergence among pool complexes separated by only tens of kilometers. In this brief report Bohonak did not analyze data on variation among pools, but it appears from his table 1 and figure 1 that variation among complexes and among regions is much greater than variation among pools within complexes.

Ketmaier et al. (2008) studied 15 populations of *Phallocryptus spinosa* in Europe and North Africa. Most populations were hundreds to thousands of kilometers apart. They partitioned the populations into four large-scale regions based on their results of phylogeographic and nested clade analysis (Templeton et al., 1995; Templeton, 1998). They found high levels of differentiation, with most of the variation (63%) due to differences among populations. With one exception, the only populations that shared a haplotype were about 13 km or less apart. They concluded that significant gene flow occurred only among populations that were very close together. The one exception was a haplotype that was found in three populations hundreds or thousands of kilometers apart.

McCafferty et al. (2010) examined 121 individuals of *E. vernalis* from 22 vernal pools in Massachusetts. They found no correlation between geographic distance and Φ_{ST} (a measure of genetic differentiation analogous to F_{ST} and G_{ST}). Most of their populations were tens of kilometers apart, but of three pairs separated by less than 1 km, two showed high levels of divergence.

Aguilar (2011) studied the COI region in 25 populations (107 individuals) of *Branchinecta lynchi* distributed throughout the central valley of California and southern Oregon. He found 39 haplotypes in four weakly supported clades, but no strong geographic pattern. Distances among populations were not given, but of seven pairs of populations nearest one another (same city in his map), five shared zero haplotypes; however, sample sizes were very small. He also found evidence of long-distance dispersal between populations in central California and southern Oregon.

Vanschoenwinkel et al. (2011) studied *Branchipodopsis* cf. *wolfi*, an undescribed species closely related to *B. wolfi*, on inselbergs (isolated rocky outcrops on mountaintops) in South Africa. They looked at genetic variation at three scales: among inselbergs (six), among pool clusters within inselbergs (four clusters on one inselberg), and among populations within clusters (nine populations in one cluster). Distances between inselbergs ranged from about 10 to 120 km; distances between clusters was usually <1 km; distances between pools within clusters was <100 m. They found 21 haplotypes, of which 76% were on one inselberg only, and 52% were in a single population. Differentiation among populations was high: Analysis of molecular variance showed 43% of variation among inselbergs, 15% among clusters within inselbergs, and 42% among populations within clusters. Isolation by distance was marginally significant only among inselbergs, and not at smaller scales.

Summarizing these results, there appear to be three general patterns of fairy shrimp variation: (1) low to moderate divergence (with some exceptions) among populations on a scale of a few kilometers or less; (2) much greater divergence among more distantly separated populations, but no strong relationship between geographic distance and genetic divergence; (3) evidence of rare long-distance dispersal and gene flow. *Streptocephalus texanus* appears to be consistent with these patterns, at a much smaller geographic scale.

The temporary pools inhabited by fairy shrimp occur in vastly different habitats. The vernal pools of New England are very different from vernal pools of the Central Valley of California or the potholes of the American Southwest. Consequently, it is reasonable to expect that dispersal mechanisms and levels vary. For example, Green and his colleagues have documented the importance of waterbirds in dispersal of propagules of a

variety of aquatic invertebrates, although they found few branchiopod cysts (Frisch et al., 2007; Green et al., 2008; Brochet et al., 2010). Bohonak and Whiteman (1999) estimated that thousands of *Branchinecta coloradensis* eggs were transported per year among a complex of mountain ponds by salamanders, *Ambystoma tigrinum*, and that about 0.9% of these eggs were viable. This corresponds well to estimates of gene flow based on allozyme F_{ST} (Bohonak, 1998).

It is frequently assumed that wind dispersal of propagules of aquatic invertebrates is inefficient and effective only over short distances (e.g. Brendonck and Riddoch, 1999; Vanschoenwinkel et al., 2009). Field studies generally confirm this. Several studies have trapped airborne propagules of branchiopods. Brendonck and Riddoch (1999) trapped only a few *B. wolfi* cysts over 3 days and suggested that the importance of wind dispersal of cysts was overestimated and important only over very short distances. Vanschoenwinkel et al. (2008*a*, 2008*b*, 2009) studied a cluster of rock pools on an isolated mountaintop in South Africa. Their general conclusions were: (1) airborne dispersal of aquatic invertebrates is common and frequent, even over short timescales; (2) they speculated that long-distance wind dispersal is not necessarily negligible over long time periods; (3) there was a sharp dropoff in the number of propagules collected beyond about 10–20 m from the nearest pool; (4) distance from the nearest dry pool was the only significant predictor of the number of propagules collected; (5) in the community they studied, dispersal by overflow among pools was very high during the rainy season, and dispersal by amphibians was far less important than by either water or wind.

Various dispersal mechanisms will result in different dispersal distances and levels, and can contribute to different patterns of geographic variation. However, they do not appear to be important in populations of *S. texanus* in the region we studied. Graham and Wirth (2008), in a wind tunnel experiment, isolated windblown cysts from LBP. They found 327 cysts from six large branchiopod species, including *S. texanus*, in only six 5-min trials. They reasoned that since windstorms in the area often last for hours, or even days, large numbers of cysts are likely blown out of potholes and potentially transferred to others. They argued that most potholes in the region lack aquatic connections, and that they are rarely visited by vertebrates (Graham reported seeing one shorebird at one pothole and great blue heron tracks in a different pothole in 18 years.) They concluded that wind dispersal is the most important mechanism of dispersal of brachiopod cysts in this region.

Our data and field observations reinforce that conclusion. We inferred moderate gene flow among MV pools and among SF pools, but found no evidence of gene flow between MV and SF. There are no aquatic connections between pools in the current study, eliminating spillover during storms as a dispersal mechanism. In addition to the birds mentioned above, ravens occasionally visit the pools to feed on tadpole shrimp (Graham, pers. observ.). It is conceivable that they pick up fairy shrimp cysts on their feet and transfer them to another pothole. However, this must be rare compared with dispersal during windstorms. The red spotted toad (*Bufo punctatus*) breeds in the potholes on the SF and MV fins, but the adults are typically in the potholes only the first night after significant summer rains to breed, and spend the rest of their time off the fins. Other amphibians have not been seen near the SF or MV potholes (Graham, pers. observ.).

Might wind direction be a consistent factor in fairy shrimp dispersal? Our only wind data came from a weather station about 26 km from the study site. Wind speed is typically (~70%) 3–16 kph but direction is quite variable, never more than 13% from any of the eight principal compass points. Thus, given the distance from the study site and the variable topography, we are unable to draw any conclusions about possible directional effects of wind dispersal.

Both genetic data and field observations are consistent with the hypothesis that wind is the most important dispersal mechanism of *S. texanus* cysts. Our results suggest that short-distance dispersal and gene flow are relatively common, but that 1 km might be enough to greatly reduce gene flow among *S. texanus* populations in this environment. However, we also see evidence of gene flow over longer distances. Additional studies are needed to assess the mechanisms and importance of medium- and long-distance dispersal in *S. texanus*.

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