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Genetic variation in the Desert Springsnail (*Tryonia porrecta*): implications for reproductive mode and dispersal

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Abstract

Allozymes and mitochondrial cytochrome *c* oxidase subunit I (*mtCOI*) sequences were analysed to determine whether populations of the western North American gastropod *Tryonia porrecta* (from California, Nevada, Utah, and northwest Mexico) are strongly differentiated in accordance with traditional interpretation of regional fauna as ancient relicts inhabiting isolated fragments of late Tertiary palaeodrainages. These data were also used to assess whether this species, for which males have not been recorded, is a rare example of a molluscan parthenogen. Both data sets strongly supported monophyly of *T. porrecta* populations. Five of the nine sampled populations consisted of a single monoallelic allozyme genotype while the others contained two to 10 distinct genotypes. Allozymic data for genetically diverse Utah populations provided evidence of clonal and sexual reproduction. *mtCOI* haplotypes of *T. porrecta* formed two subgroups which differed by 1.99–2.60%. The common haplotype was found in seven populations with rare haplotypes observed in single populations. Based on these results and an available *mtCOI* molecular clock for related hydrobiid snails, *T. porrecta* is interpreted as a primarily parthenogenetic species that undergoes occasional sexual reproduction and has accumulated substantial diversity following its mid-Pliocene to mid-Pleistocene origin. Our results also suggest that the distribution of present-day populations of these gill-breathing snails did not result from fragmentation of an ancient, well-integrated drainage but instead reflects overland colonization of habitats which only recently became available following desiccation of late Quaternary pluvial lakes.

Keywords: allozymes, biogeography, Gastropoda, mtDNA, parthenogenesis, *Tryonia*

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'Series of springs in desert regions form aquatic archipelagos that differ from their oceanic analogs in that they often contain certain organisms that are relicts of past ages, rather than organisms resulting from chance invasion and subsequent differentiation'. (Minckley & Deacon 1968: 1425)

Introduction

The southwestern North American deserts contain a diverse and locally abundant fauna of gill-breathing gastropods. As with other regional aquatic biota (e.g. Polhemus & Polhemus 2002; Smith *et al.* 2002), the distribution and evolutionary history of these snails is thought to be closely linked with regional drainage history based on an assumption that these animals are incapable of overland dispersal and spread only through their habitats (Taylor 1985; Taylor & Bright 1987). The present-day members of this gastropod fauna are considered relicts whose distributions trace Neogene drainages that were fragmented by subsequent changes in climate and landscape (Taylor 1964, 1985, 1987). Although the assumptions and implications of this pervasive biogeographical model are amenable to testing using the tools of molecular genetics, the western aquatic gastropods are only beginning to be studied in this manner. One such study of hydrobiid snails (genus *Pyrgulopsis*) documented extensive divergence among populations of a widespread species consistent with this biogeographical model (Liu *et al.* 2003), while another provided surprising evidence of gene flow over large distances (Hershler & Liu 2004a, b).

The genus *Tryonia* (family Cochliopidae) includes 15 species which live in this desert region (Hershler 2001).

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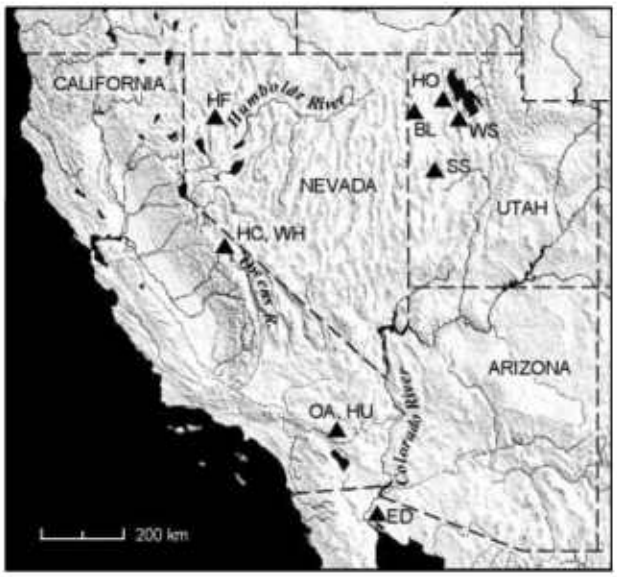


Fig. 1 Map showing location of sampled populations of *Tryonia porrecta* in the Great Basin. BL, Blue Lake; ED, El Doctor; HC, Hot Creek; HF, Hualapai Flat; HO, Horseshoe Springs; HU, Hunters Spring; OA, Oasis Spring; SS, South Spring; WH, Whitmore Hot Springs; WS, Warm Springs.

These tiny aquatic snails have an entirely benthic life cycle; sexes are separate and young are brooded in the female genital duct (Hershler 2001). Fourteen of the 15 species are endemic to single springs or local spring systems (Hershler 2001), consistent with their presumably limited vagility and long period of isolation. In contrast, *Tryonia porrecta* (*T. protea* of authors; Hershler 2001) consists of widely scattered groups of populations within the Great Basin (Hershler 2001; Fig. 1). Taylor (1985) interpreted a portion of this species' range as evidence of a Pliocene or older surface water connection between the Bonneville (Utah) and lower Colorado River basins. The remaining portion of this uniquely disjunct distribution spans diverse topography and also suggests geologically ancient (late Tertiary) drainage connections. [Taylor (1987) suggested that extant congeners are of Miocene age.] If the broadly disjunct populations of *T. porrecta* are relicts that have long been isolated from one another in accordance with the traditional biogeographical model, then substantial genetic divergence is expected. However, recent surveys of genetic variation documented only limited mtDNA sequence divergence and allozymic uniformity of several populations of this species (Hershler *et al.* 1999a, b). Hershler *et al.* (1999b) suggested that *T. porrecta* is a parthenogen whose broad distribution reflects increased dispersal ability associated with asexual reproduction rather than historical drainage connections. Parthenogenesis is an extremely rare reproductive mode in the phylum Mollusca (Suomalainen *et al.* 1987; Dillon 2000) and has not been previously reported for any other native member of the western North American fauna.

In the present study, we use allozymes and partial DNA sequences for the mitochondrial cytochrome *c* oxidase subunit I gene (*mtCOI*) to more fully describe variation in *T. porrecta* throughout its western range and assess the genetic structure, divergence, and phylogenetic relationships of its populations. We use these results to further evaluate whether *T. porrecta* reproduces parthenogenetically and to explore the biogeographical history of this unusual snail.

Materials and methods

Tryonia porrecta was sampled from each of the four widely separated western areas [Bonneville basin (western Utah), Salton Trough (southeast California, northwest Mexico), Lahontan basin (western Nevada), Owens River basin (southeast California)] in which it occurs (Fig. 1). In those areas in which these snails range among multiple localities, two or more populations were sampled. Snails were either frozen in the field and stored at –70 °C or directly preserved in concentrated (90%) ethanol. Voucher specimens were reposited at the Smithsonian's National Museum of Natural History.

Monophyly of *T. porrecta* populations was tested in the allozyme analysis by inclusion of three regional congeners (*Tryonia imitator*, *Tryonia margae*, *Tryonia salina*) that were previously hypothesized to be closely related to this species (Hershler *et al.* 1999b) and a more distantly related congener, *Tryonia clathrata*. For the analysis of *mtCOI* sequences, a broader spectrum of congeners was sampled including four undescribed species from northern Mexico (R. Hershler & H.-P. Liu, in preparation). The hypothesized sister group of *Tryonia*, monotypic *Mexipyrgus*, was used to root these trees (Hershler *et al.* 1999b). Locality data and GenBank Accession nos are given in Table 1.

Protein electrophoresis

Whole snails were homogenized in 20-μL grinding buffer (0.01 M Tris, 0.001 M EDTA, 0.5 mM NADP, pH 7.0) using a glass rod. Homogenate fluid was absorbed onto filter paper wicks and inserted into 12% horizontal starch gels. The following combinations of buffer and enzymes were used: Tris-citrate, pH 8.0 (Selander *et al.* 1971) for glucose-6-phosphate isomerase (*GPI*, EC 5.3.1.9), phosphogluconate dehydrogenase (*PGDH*, EC 1.1.1.44) and malate dehydrogenase (NADP+) (*MDHP*, EC 1.1.1.40); lithium hydroxide, pH 8.1 (Selander *et al.* 1971) for mannose-6-phosphate isomerase (*MPI*, EC 5.3.1.8) and purinenucleoside phosphorylase (*NP*, EC 2.4.2.1); Tris-citrate-EDTA, pH 7.1 (Ayala *et al.* 1972) for isocitrate dehydrogenase (*IDH*, EC 1.1.1.42); and Tris-borate-EDTA, pH 8.0 (Selander *et al.* 1971) for phosphoglucomutase (*PGM*, EC 5.4.2.2), aspartate aminotransferase (*AAT*, EC 2.6.1.1), and esterase (*EST*, EC 3.1.1.1) with 4-methylumbellyliferyl acetate as substrate.

Table 1 *Tryonia* samples with codes, localities, and GenBank Accession nos (*N* = sample size)

Species	Code	Locality	GenBank Accession nos
Tryonia porrecta	WH	Whitmore Hot Springs, Long Valley, Owens River basin, Mono Co., CA	AF061773* (*N* = 3)
	HC	Hot Creek, Long Valley, Owens River basin, Mono Co., CA	AY803024 (*N* = 3)
	OA	Oasis Spring, Salton Sea basin, Riverside Co., CA	AF061772* (*N* = 1)
	HU	Hunters Spring, Salton Sea basin, Riverside Co., CA	AY803025 (*N* = 4)
	SS	South Springs, Fish Springs Flat, Bonneville basin, Juab Co., UT	AY803023, AY803029, AY803030, AY803032 (*N* = 6)
	HO	Horseshoe Springs, Skull Valley, Bonneville basin, Tooele Co., UT	AF129312†, AY803027, AY803028, AY803032 (*N* = 4)
	WS	Warm Springs, Tooele Valley, Bonneville basin, Tooele Co., UT	AF129313† (*N* = 2)
	BL	Spring, Blue Lake, Bonneville basin, Tooele Co., UT	AF129314† (*N* = 2)
	HF	Spring, Hualapai Flat, Lahontan basin, Washoe Co., NV	AY803033, AY803034 (*N* = 5)
	ED	Spring at El Doctor, Colorado River basin, Sonora, Mexico	AY803026 (*N* = 3)
Tryonia aequicostata	AS	Alexander Springs, St Johns River basin, Lake Co., FL	AF129302†
	LE	Lake Eustis, St Johns River basin, Lake Co., FL	AF129301†
Tryonia cheatumi		Phantom Lake Spring, Pecos River drainage, Jeff Davis Co., TX	AF129305†
Tryonia circumstriata	DY	Diamond Y Spring, Pecos River drainage, Pecos Co., TX	AF129306†, AF129307†
	DD	Diamond Y Draw, Pecos River drainage, Pecos Co., TX	AF129308†
Tryonia clathrata	MV	Warm Spring, Moapa Valley, Clark Co., NV	AF061767*
	AS	Ash Spring, Pahranagat Valley, Lincoln Co., NV	
Tryonia imitator	PQ	Penasquitos Lagoon, San Diego Co., CA	AF061769*
	MC	Moro Cojo Lagoon, Monterey Co., CA	AF061770*
Tryonia margae	G1	Grapevine Springs (cool spring), Death Valley, Inyo Co., CA	
	G2	Grapevine Springs (lower warm spring), Death Valley, Inyo Co., CA	
	G3	Grapevine Springs (upper warm spring), Death Valley, Inyo Co., CA	AF061771*
Tryonia monitorae		Spring, Potts Ranch, Monitor Valley, Nye Co., NV	AF129316†
Tryonia quitobaquitae		Quitobaquito Spring, Rio Sonoyta basin, Pima Co., AZ	AF128315†
Tryonia salina		Cottonball Marsh, Death Valley, Inyo Co., CA	AF061776*
Tryonia n. sp. 1		Spring, Nuevo Casas Grandes, Rio Casas Grandes basin, Chihuahua, Mexico	AY803035, AY803036
Tryonia n. sp. 2		Spring, northeast of Zaragoza, internal drainage, Chihuahua, Mexico	AY803038
Tryonia n. sp. 3		Spring, southeast of Galeana, Rio de Santa Maria basin, Chihuahua, Mexico	AY803037
Tryonia n. sp. 4		Spring at Talamantes, Rio Florida basin, Chihuahua, Mexico	AY803039
Mexipyrgus carranzae		Mojarral West Laguna, Cuatro Cienegas basin, Coahuila, Mexico	AF129325†

*Hershler *et al.* 1999a; †Hershler *et al.* 1999b.

Gels were stained using methods outlined in Selander *et al.* (1971) and Richardson *et al.* (1986). Specimens from several populations were run concurrently on all gels to facilitate comparison of electrophoretic mobilities. Two specimens of *T. porrecta* from Whitmore Hot Springs were included on all gels to serve as a reference for allozyme mobilities. Mobilities for the alleles of this reference population were arbitrarily designated 100 and other alleles named relative to this standard. Allozyme data for outgroup species and California samples of *T. porrecta* are from Hershler *et al.* (1999a).

Allelic frequencies, number of polymorphic loci, mean number of alleles, and mean heterozygosity were calculated using BIOSYS-1 (Swofford & Selander 1981). Polymorphic allozyme loci were also tested for conformance to Hardy–Weinberg proportions using this software while multilocus genotypes were tested for linkage disequilibrium using GENEPOP, option 2 (http://wbiomed.curtin.edu.au/genepop/genepop_op2.html; adapted from Raymond & Rousset 1995) (Markov chain parameters: 5000 dememorization steps, 500 batches, 5000 iterations per batch). GENDIST and KITSCH from PHYLIP (Felsenstein 1993) were used to calculate genetic distance (Nei 1972) and generate trees. KITSCH uses the Fitch–Margoliash and least-squares-distance methods but produces a tree typology comparable to the UPGMA method as it assumes that a constant or equal rate of change is operative along all tree branches. Topological confidence was evaluated with 1000 bootstrap replicates using SEQBOOT and CONSENSE in the PHYLIP package.

DNA sequences

Genomic DNA was isolated from whole snails using a Chelex extraction (Walsh *et al.* 1991). The *mtCOI* gene was

amplified using primers *COIL490* (5′-GGTCAACAAATCATAAAGATATTGG-3′) and *COIH2198* (5′-TAAACTTCAGGGTGACCA AAAAATCA-3′) (Folmer *et al.* 1994). Approximately 100 ng of genomic DNA was used as template for double-stranded polymerase chain reactions (PCRs). For 50-μL reactions, we used each dNTP at 250 μM, each primer at 0.5 μM, 1 unit *Taq* polymerase (Promega), and 10 μL of 5× optimizer buffer F (Invitrogen). Reactions were amplified for 33 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min. Double-stranded DNA products were purified with Microcon-100 filters. Purified products were used as templates for automated sequencing following Applied Biosystems protocol.

Sequences were proofed and aligned using SEQUENCHER. Alignments were verified by eye. Mutational saturation for each codon was examined by plotting the absolute number of transitions and transversions against inferred genetic distance (TrN distance). No mutational saturation was evident for all three codon positions. The TrN model with variable sites assumed to follow a discrete gamma distribution (e.g. TrN + Γ; Tamura & Nei 1993) was selected as the best fit for the data set using hierarchical likelihood ratio tests in MODELTEST 3.06 (Posada & Crandall 1998). Note, however, that Posada & Buckley (2004) recently suggested that the Akaike information criterion (AIC) and Bayesian methods offer advantages for model selection.

Phylogenetic hypotheses based on distance, parsimony, and maximum-likelihood (ML) methods were conducted using PAUP* version 4.0b10 (Swofford 2002). Maximum-parsimony (MP) analyses were conducted with equal weighting, using the heuristic search option with tree-bisection–reconnection (TBR) branch-swapping and 100 random additions. Bootstrapping with 10 000 replications (as implemented in PAUP*) was used to evaluate node support. Tamura-Nei (TrN) distances were used to generate a neighbour-joining (NJ) tree based on the clustering method of Saitou & Nei (1987). Node support was assessed by completion of 1000 bootstrap replications (Felsenstein 1985) in PAUP*, using the fast-search option. ML analyses were based on the TrN + Γ model using the heuristic search algorithm. An NJ tree (TrN distances) was used as the initial topology for branch-swapping. Node support was evaluated by 100 bootstrap pseudoreplicates. Random trees were generated in PAUP and skewness (g_1) statistics were used to evaluate whether the data set contained significant phylogenetic information (Hillis & Huelsenbeck 1992).

Results

Allozymes

Allozyme phenotypes were typical of those observed in diploid species, for example there was no asymmetry of stain intensity as reported for the triploid gastropod *Potamopyrgus antipodarum* (Fox *et al.* 1996). Four of the nine enzyme loci exhibited polymorphism in *Tryonia porrecta* samples. The number of polymorphic loci per population ranged from zero (California and Nevada samples) to four (South Springs, Horseshoe Springs). Mean heterozygosity varied from 0% (as above) to 22.6% for Warm Springs. The mean number of alleles per locus ranged from 1.0 (California and Nevada samples) to 1.6 for Horseshoe Springs. Genotypic frequencies in populations are summarized in Table 2. No relationship between sample size and number of allozyme genotypes was observed.

Samples from California and Nevada were monoallelic (homozygous) at all loci, and monomorphic for a single multilocus genotype whereas those from Utah consisted of two (Blue Lake, Warm Springs), five (Horseshoe Springs), and 10 (South Springs) genotypes (Table 2, Fig. 2A). The *IDH* 100/80 genotype was found only in the Horseshoe Springs sample. Fixed heterozygous genotypes were observed for *GPI* in the Blue Lake and Warm Springs samples and for *PGDH* in the Warm Springs sample. Samples from South Springs and Horseshoe Springs had the greatest allozyme diversity in terms of alleles per locus, and multilocus genotypes.

There were six significant departures from Hardy–Weinberg expectations out of 13 possible combinations of population and loci. Genotype frequencies which significantly departed from Hardy–Weinberg expectations were observed in the Blue Lake population at the *GPI* locus, Warm Springs at the *GPI* and *PGDH* loci, and Horseshoe Springs at the *GPI*, *PGDH*, and *PGM* loci (Table 3). In most but not all cases (11/13) departures from Hardy–Weinberg expectations were caused by heterozygote excess. A pairwise test for linkage disequilibrium across all populations provided six possible comparisons. Five of six of these tests showed a significant association between loci (using the standard Bonferroni correction, the *P* value was lowered to 0.008).

In the KITSCH (UPGMA) tree (Fig. 3), *T. porrecta* populations formed a well-supported (95%) clade and clustered together at Nei distances less than 0.06. This clade was depicted (with weak bootstrap support) as sister to *Tryonia imitator* (a Pacific coastal species) and was subdivided into moderate to well-supported California–Nevada (95%) and Utah (61%) subclades. Distance values between *T. porrecta* and other congeners ranged from 0.445 (*T. imitator*) to 0.895 (*Tryonia clathrata*).

mtCOI

Thirty-three new sequences were obtained for this study. Seventeen of these were deposited in GenBank under Accession nos AY803023–AY803039 (Table 1) (only one sequence for each haplotype from each population was

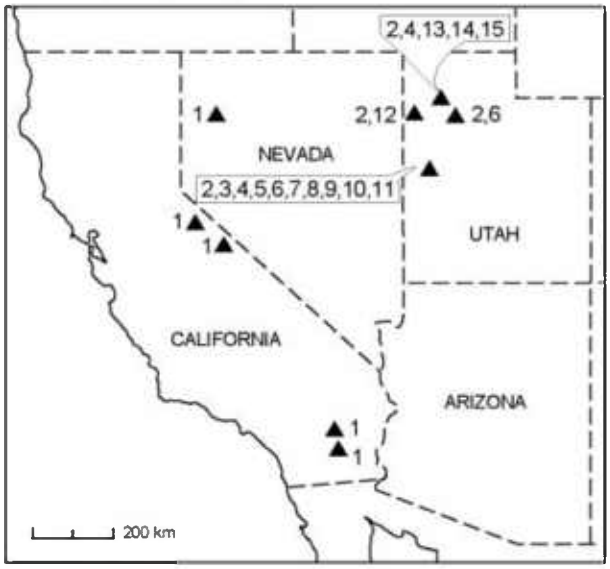
Table 2 Distribution of allozyme genotypes in populations of *Tryonia porrecta*. Five additional loci were monoallelic in all populations. Sample sizes are indicated in parentheses and frequency of multilocus genotypes in each population is listed on the right

		GPI		*PGDH*			*PGM*			*IDH*			
Population	Genotype	100/100	130/100	100/100	84/84	100/84	100/100	85/85	100/85	100/100	110/100	100/80	Frequency
Whitmore Hot Springs (WH) (> 50)	1	X		X			X			X			1.00
Hot Creek (HC) (30)	1	X		X			X			X			1.00
Hunters Spring (HU) (24)	1	X		X			X			X			1.00
Oasis Spring (OA) (20)	1	X		X			X			X			1.00
Hualapai Flat (HF) (44)	1	X		X			X			X			1.00
South Springs (SS) 28	2		X			X	X			X			0.08
	3	X				X			X		X		0.12
	4	X		X				X			X		0.08
	5	X			X		X			X			0.20
	6		X			X			X	X			0.20
	7	X				X		X			X		0.04
	8	X			X		X				X		0.08
	9	X		X					X		X		0.08
	10	X				X		X		X			0.04
	11	X				X	X				X		0.08
Blue Lake (BL) (32)						X	X			X			0.28
	2		X										
	12		X		X		X			X			0.72
Horseshoe Springs (HO)20	2		X			X	X			X			0.50
	4	X		X				X			X		0.20
	13	X				X	X			X			0.15
	14		X	X			X			X			0.05
	15		X			X	X					X	0.10
Warm Springs (WS) (30)	2		X			X	X			X			0.97
	6		X			X			X	X			0.03

deposited). The total aligned DNA data consisted of 653 base pairs (bp) of the *mtCOI* gene. There were 139 variable sites and 95 sites were parsimony informative. The distribution of 10 000 randomly generated trees was significantly skewed ($g_1 = -0.37$, $P < 10^{-3}$), suggesting that this data set was phylogenetically informative. All phylogenetic analyses of the *mtCOI* data set resolved a strongly supported *T. porrecta* clade (90–100% bootstrap support) (Fig. 4, a ML tree). A sister relationship between *T. porrecta* populations and a clade composed of a southern Arizona species (*Tryonia quitobaquitae*) and four undescribed congeners from northern Mexico was resolved in all analyses but not well supported by bootstrap values (< 50%). In all trees *T. porrecta* sequences formed two moderate to well-supported (63–100%) subclades. One clade contained specimens from Nevada and Utah, while the other was composed of specimens from California, Mexico, and Utah.

The MP analysis yielded two equally parsimonious trees (TL = 239, CI = 0.63) which differed in relative positions of terminals within the *T. porrecta* clade. The MP results differed from the ML topologies in the position of *T. clathrata*, which was basal instead of as in Fig. 4, and in the relative positions of *T. salina* and *T. imitator* within their subclade. The NJ tree differed from the ML topology only in the relative positions of *T. salina* and *T. imitator* within their subclade.

A. Allozyme genotypes



B. *COI* haplotypes

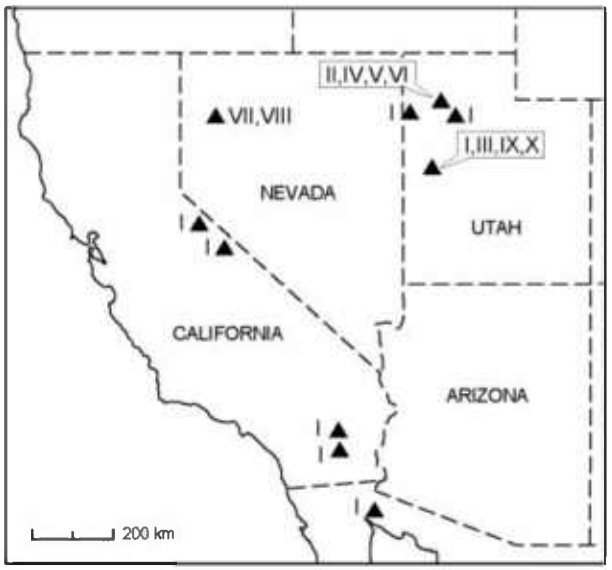


Fig. 2 Geographic distribution of genetic diversity of *Tryonia porrecta*. A. Allozyme genotypes, labelled as in Table 2. B. *COI* haplotypes, labelled as in Table 4. Note that allozyme data are not available for the El Doctor population (northwest Mexico).

Table 3 Statistical comparison of genotypic frequencies at polymorphic loci with those expected at Hardy–Weinberg equilibrium (HWE). χ^2, chi-squared value for deviation from HWE. *P*, significance level of probability of fit to HWE. *D*, heterozygote deficiency/excess

		Allozyme locus			
Sample		*GPI*	*PGDH*	*PGM*	*IDH*
South	χ^2	0.710	0.629	0.578	2.79
Springs (SS)	*P*	0.399	0.428	0.447	0.096
	D	0.154	0.145	−0.139	0.304
Blue	χ^2	32.000	0.857		
Lake (BL)	*P*	0.000	0.355		
	D	1.000	0.164		
Horseshoe	χ^2	4.636	7.200	20.000	0.623
Springs (HS)	*P*	0.031	0.007	0.000	0.891
	D	0.481	0.600	−1.000	0.132
Warm	χ^2	30.000	30.000	0.009	
Springs (WS)	*P*	0.000	0.000	0.926	
	D	1.000	1.000	0.017	

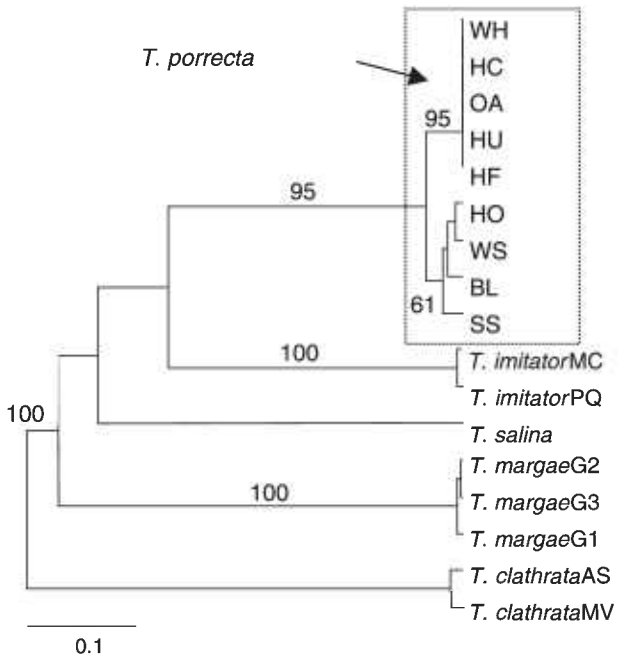


Fig. 3 UPGMA tree (based on Nei genetic distance) generated from allozyme data for *Tryonia porrecta* and related congeners. Locality codes as in Table 1. Numbers indicate bootstrap support.

Within samples of *T. porrecta*, sequence divergence ranged from 0% (Blue Lake, El Doctor, Hot Creek, Hunters Spring, Oasis Spring, Warm Springs, Whitmore Hot Springs) to 2.5% for Horseshoe and South Springs. Sequence divergence was greater than 4.6% for all comparisons between *T. porrecta* and its congeners. For interspecific comparisons among congeners except *T. porrecta*, sequences differed by 1.7–7.0%.

Twenty-three variable sites were found among the *T. porrecta* samples for a total of 10 *mtCOI* haplotypes (Table 4). Haplotype relationships are illustrated in a network in Fig. 5. Haplotypes formed two groups which differed from one another by 13 or more bp changes. Haplotype I was the most common and widely distributed (Fig. 2B) and differed from other members of its group

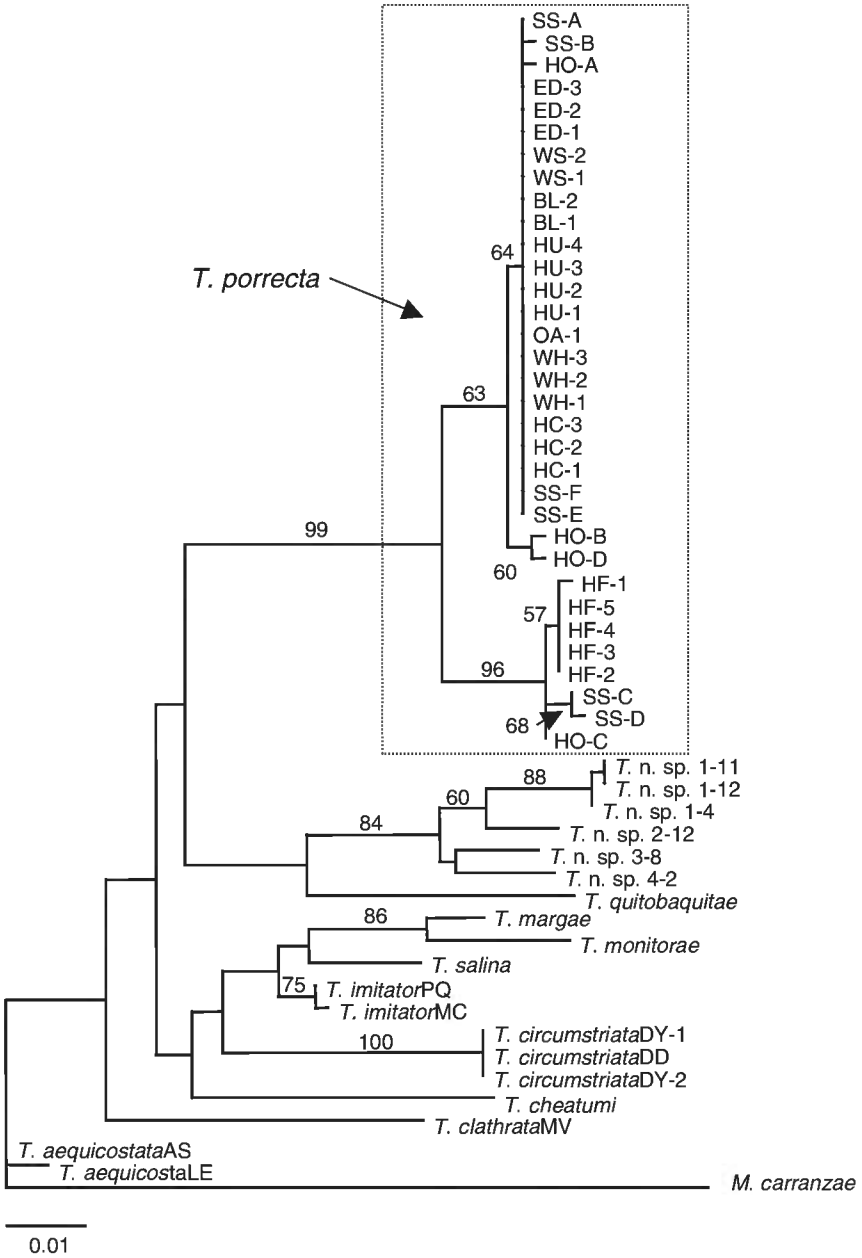


Fig. 4 One of two ML trees generated from *mtCOI* sequence data for *Tryonia porrecta* and related congeners.sequences. Locality codes as in Table 1. Numbers indicate bootstrap support.

(II–V) by one or four bp changes. Within the other group, haplotypes VII–X differed from haplotype VI by 1 to 3 bp changes. Populations at Horseshoe and South Springs contained highly divergent representatives of both haplotype groups. For example, haplotypes III and IX from the South Springs population differed by 16 bp.

Discussion

Genetic diversity and reproductive mode

Tryonia porrecta has been assumed to be uniformly parthenogenetic based on the absence of records of males for this species (Hershler 2001). These snails are easily sexed as males are phallate and females brood large young in their genital duct (Hershler 2001). Populations consisting of one or two multilocus allozyme genotypes readily fit this interpretation, as does the occurrence of fixed heterozygous genotypes and allozymic evidence of significant linkage disequilibrium in other populations (*fide* Tibayrenc *et al.* 1991). However, populations such as that of South Springs which exhibited high genotypic diversity and genotype frequencies fitting random mating expectations are more difficult to explain.

Although parthenogenesis has been previously inferred (based on absence of male records) for several other species

Table 4 Mitochondrial cytochrome c oxidase subunit I haplotypes in *Tryonia porrecta* populations. Numbers indicate positions along the 653-bp segment

Haplotype	9	47	56	63	110	167	176	183	207	248	299	306	335	342	348	358	364	375	423	437	555	569	653
I	T	G	A	G	T	G	G	G	C	G	G	A	T	C	A	C	C	A	T	T	C	C	T
II	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
III	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
IV	.	A	G	.	.	.	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.
V	.	.	G	.	.	.	.	.	.	A	.	.	.	.	G	.	G	.	.	.	.	.	.
VI	C	.	.	A	C	A	.	.	.	.	A	.	C	T	G	T	.	G	C	.	.	T	C
VII	C	.	.	A	C	A	.	.	.	.	A	G	C	T	G	T	.	G	C	.	.	T	C
VIII	C	.	.	A	C	A	.	.	.	.	A	G	C	T	G	T	.	G	C	.	T	T	C
IX	C	.	.	A	C	A	A	.	.	.	A	.	C	T	G	T	.	G	C	C	.	T	C
X	C	.	.	.	C	A	A	.	.	.	A	.	C	T	G	T	.	G	C	C	.	T	C

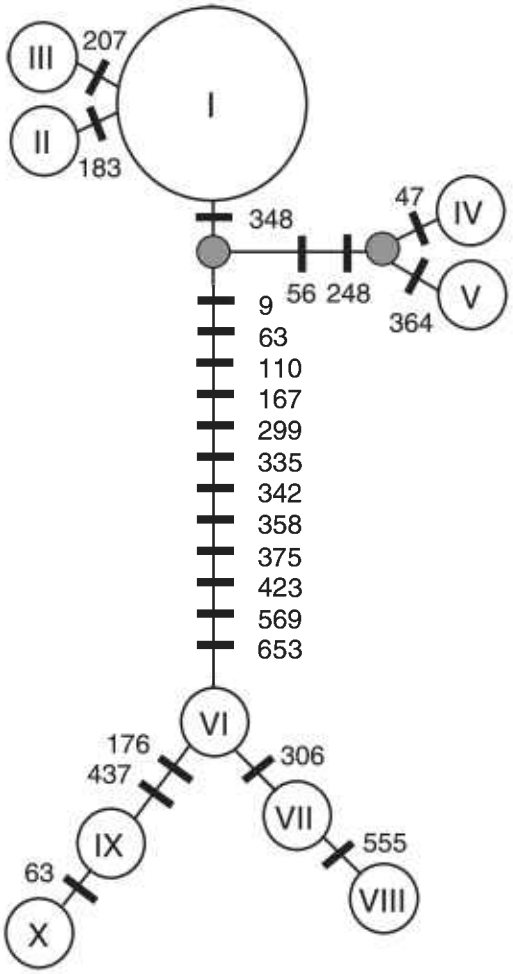


Fig. 5 Network for the 10 haplotypes of *Tryonia porrecta mtCOI* sequence. Filled dots refer to unobserved haplotypes that are inferred to have existed in this species. Numbers refer to bp positions at which changes occurred.

of the family Cochliopidae (Giusti & Pezzoli 1984; Martín 2002), there have been no pertinent genetic studies of these taxa. However, comparable allozyme studies of other aquatic snails suggest several possible mechanisms for the generation of genetic diversity within parthenogens. Triploid populations of *Potamopyrgus antipodarum* in New Zealand consist of numerous clones which were apparently derived from sympatric congeners by repeated mutation to parthenogenetic reproduction (Dybdahl & Lively 1995). Diverse clonal populations of the eastern North American genus *Campeloma* were also iteratively evolved through spontaneous mutations, as well as by hybridization of sexual species (Johnson 1992; Johnson & Leefe 1999). Inasmuch as populations of *T. porrecta* form a strongly supported, morphologically cohesive (Hershler 2001) clade that is well differentiated and widely separated geographically relative to other members of the genus, their genetic diversity cannot be readily attributed to multiple origins from sexual congeners. Also note that a comparable suite of single-locus allozyme genotypes was found in all populations of *T. porrecta*, which is most readily attributed to a single origin of these parthenogens. (Note that the *IDH* 100/80 genotype was rare and limited to a single population.) Populations exhibiting fixed heterozygous genotypes are often observed in parthenogens which arose through hybridization of sexual species. However, heterozygous genotypes of *T. porrecta* were not uniformly distributed across loci or clones as would be expected if this species originated as an interspecific hybrid. The genetic structure of *T. porrecta* also does not conform to the simple model of mutational divergence following population isolation proposed for an Australian parthenogen (*Thiara balonnensis*), which consists of unique clonal genotypes at each locality (Stoddart 1983).

Perhaps the most likely explanation for the genetic structure of *T. porrecta* is that this primarily parthenogenetic snail occasionally undergoes sexual reproduction, which even at very low frequencies can generate substantial diversity in the nuclear genome and the appearance of panmixia (Bengtsson 2003). Livshits *et al.* (1984; also see Livshits & Fishelson 1983) favoured this mechanism to explain allozyme variation in parthenogenetic populations of the freshwater snail *Melanoides tuberculata* in Israel (but

see Samadi *et al.* 1998). The discovery of functional *T. porrecta* males in the genetically diverse Utah populations would corroborate this hypothesis.

Evolutionary and biogeographical considerations

The occurrence of broadly disjunct yet genetically homogeneous populations of *T. porrecta* (Fig. 2) conflicts with the traditional biogeographical model for western aquatic mollusks. This model also conflicts more generally with *mtCOI* sequence divergence data suggesting a relatively recent origin of this species. *T. porrecta* is composed of two divergent and geographically overlapping groups of haplotypes which differed by 2.2 ± 0.52% (13–17 bp changes; Fig. 5). The divergence between these two haplotype groups suggests a minimum age of 1.20 ± 0.28 million years (Myr) for the species based upon a previously derived, geologically calibrated *mtCOI* clock estimate of 1.83% per Myr for the closely related Hydrobiidae (Wilke 2003). The maximum age of *T. porrecta* can be similarly estimated by comparison with its congeners. Inasmuch as the sister species of *T. porrecta* has not yet been confidently identified, we performed this calculation using both *T. imitator*, which is closest to *T. porrecta* based on the allozyme results (Fig. 3) and *mtCOI* genetic distance (5.0 ± 0.9%), and *T. quitobaquitae* (6.4 ± 1.0%), which is depicted as a close relative in the *mtCOI* tree (Fig. 4). The sequence divergence between *T. porrecta* and these two congeners suggests a maximum age of 2.73 ± 0.49 Myr to 3.50 ± 0.55 Myr. Note in this context that the hypothesized prior connection between the Bonneville and lower Colorado River basins, which was based in part on the distribution of *T. porrecta*, was estimated to have occurred 6–5 million years ago (Ma) (well prior to the origin of *T. porrecta*) based on molecular similarity of fish taxa (Dowling *et al.* 2002). The biogeographical pattern of *T. porrecta* implies dispersal well subsequent to the hypothesized occurrence and break-up of a regionally integrated late Tertiary drainage, and is consistent with geological evidence that modern populations of these snails were only recently founded. Populations in Long Valley (Hot Creek, Whitmore Hot Springs) inhabit a 0.7-million-year caldera (Bailey *et al.* 1976) and must have been founded following the volcanic eruption that formed this structure. These and all other inland populations of *T. porrecta* occupy basinal sites that were inundated by deep, cold water lakes during the Late Quaternary (Lakes Bonneville, Cahuilla, Lahontan, Long Valley). It is unlikely that *T. porrecta*, which presently occupies thermal spring habitat, lived in these palaeolakes or in submarine springs within these water bodies. The modern habitats of these snails instead were likely colonized (from other areas) after desiccation of these lakes, which occurred from the late Pleistocene to as recently as 750 BP [Lake Cahuilla (Salton Trough); Waters 1983].

The apparently recent colonization of hydrographically separated habitats suggests that spread of these parthenogenetic snails has not occurred through aquatic connections but instead by overland dispersal. Perhaps these populations were founded from source areas by avian transport, as suggested by the occurrence of *T. porrecta* in wetland areas that serve as stopovers for large numbers of migrating birds (e.g. Hinojosa-Huerta *et al.* 2003; Barnum & Johnson 2004). While Taylor & Bright (1987) argued that obligately aquatic mollusks of western North America are not subject to overland transport and thus attributed their distributions instead to drainage history, they also acknowledged the possibility of passive transport of small species on insects and birds (see references cited therein and also Rees 1965; Figuerola & Green 2002). Human activities also may have enabled transport of *T. porrecta* across terrestrial barriers, as evidenced by records of presumably introduced populations of this species from a small artificial pond in Arizona (R. Hershler & J. Landye, unpublished) and Holocene deposits in Hawaii (Cowie 1997). Note that another ovoviviparous, parthenogenetic snail, *Potamopyrgus antipodarum*, has also demonstrated an ability to readily disperse across dry land (Ponder 1988).

We speculate that the ancestral sexual progenitor of *T. porrecta* was restricted in distribution (similar to other western congeners) and lived in the Bonneville basin. Indeed, a second *Tryonia*-like species was present in this region during the Pleistocene (Taylor & Bright 1987), which may be pertinent to this hypothesis. Bonneville basin populations (e.g. Horseshoe and South Springs) display the greatest amount of genetic variation, as would be expected if this represents 'frozen' ancestral diversity, and were the likely source for other populations of this species. Multiple-locus genotypes of these snails differed in number and detail among localities, which may reflect sampling of ancestral diversity and differential spread and persistence of clones over time. Genetically homogeneous populations in California presumably reflect colonization of a single successful clone. It is likely that this genetic uniformity reflects a stepping-stone pattern of dispersal rather than multiple independent events.

Populations of *T. porrecta* provide a striking exception to the biogeographical pattern exhibited by its congeners and other western North American aquatic fauna, which have apparently been long isolated by dry land barriers and consequently well differentiated genetically, resulting in the evolution of narrowly endemic species. We have argued herein that the broad geographical distribution and pattern of population divergence of *T. porrecta* may be explained in large part by its unusual, parthenogenetic mode and associated high dispersal ability. Cytological studies and a more detailed genetic survey of Utah populations will be necessary to determine the origin and type of parthenogenesis in this species, while additional collections

will be needed to confirm the presence of rare males. We have also argued that *T. porrecta* localities known to us were only recently colonized following the Late Quaternary desiccation of pluvial lakes (which would have inundated these sites). Field surveys are needed to determine whether there are (or were) any localities of this species that were not within the confines of these palaeolakes and thus could have served as sources for other modern day populations.

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This paper continues a series of collaborative studies by the authors on the molecular systematics and evolution of western North American freshwater gastropods.
