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Multiscale patterns of genetic structure in a marine snail (*Solenosteira macrospira*) without pelagic dispersal

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Abstract The northern Gulf of California (NGC) is one of the most dynamic and productive marine ecosystems in the world, yet knowledge about population connectivity and dispersal patterns is lacking for many of its resident species. Using nuclear and mitochondrial markers, we investigated the effects of open water, geographical distance and suitable habitat on patterns of genetic structure of *Solenosteira macrospira*, a benthic buccinid whelk with direct development. We collected samples in April 2004, 2005 and May 2007 from the upper NGC (31°34.39′N, 114°44.45′W). Phylogenetic analyses, hierarchical analyses of variance and Bayesian assignment tests substantiated a break between the east and west coasts. Genetic distance between population pairs increased with geographical distance, but only when assuming a U-shaped dispersal pathway over the open water of the NGC. Given *S. macrospira*'s association with rocky intertidal habitats, and its limited dispersal potential, we assumed that the geographical distribution of rocky habitat would play a significant role in genetic differentiation of *S. macrospira*. Nevertheless, populations separated by sand were more similar than populations separated by rocks. The influence of open water, geographical distance and suitable habitat (rocks vs. sand) also varied significantly across different genetic markers that presumably evolve at different rates. Specifically, the more rapidly evolving nuclear microsatellites suggested that physical transport processes strongly influence genetic differentiation on contemporary time scales, even in a species with direct benthic development. This underscores the strong, and potentially homogenizing, effect of present-day ocean circulation patterns in the NGC.

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Introduction

Sessile and sedentary marine invertebrates exhibit a range of dispersal potentials, with some species having long-lived larvae that may drift and feed for months in the ocean, and others entirely lacking a pelagic larval stage. In general, marine species with long-lived larvae and extensive dispersal potential tend to exhibit genetic structure over greater geographical ranges than species with short-lived larval phases and limited dispersal potential. Although many marine species conform to this expectation (reviewed in Bohonak 1999; Kinlan and Gaines 2003; Dawson et al. 2006; Cowen and Sponaugle 2009), a surprisingly large number of species display patterns of genetic structure that contradict predictions based primarily on estimates of pelagic larval duration (Kyle and Boulding 2000; Thorrold et al. 2002; Palumbi 2003; Marko 2004; Lester et al. 2007; Hellberg 2009; Shanks 2009; Weersing and Toonen 2009; Winston 2012). The paradox of Rockall embodies this

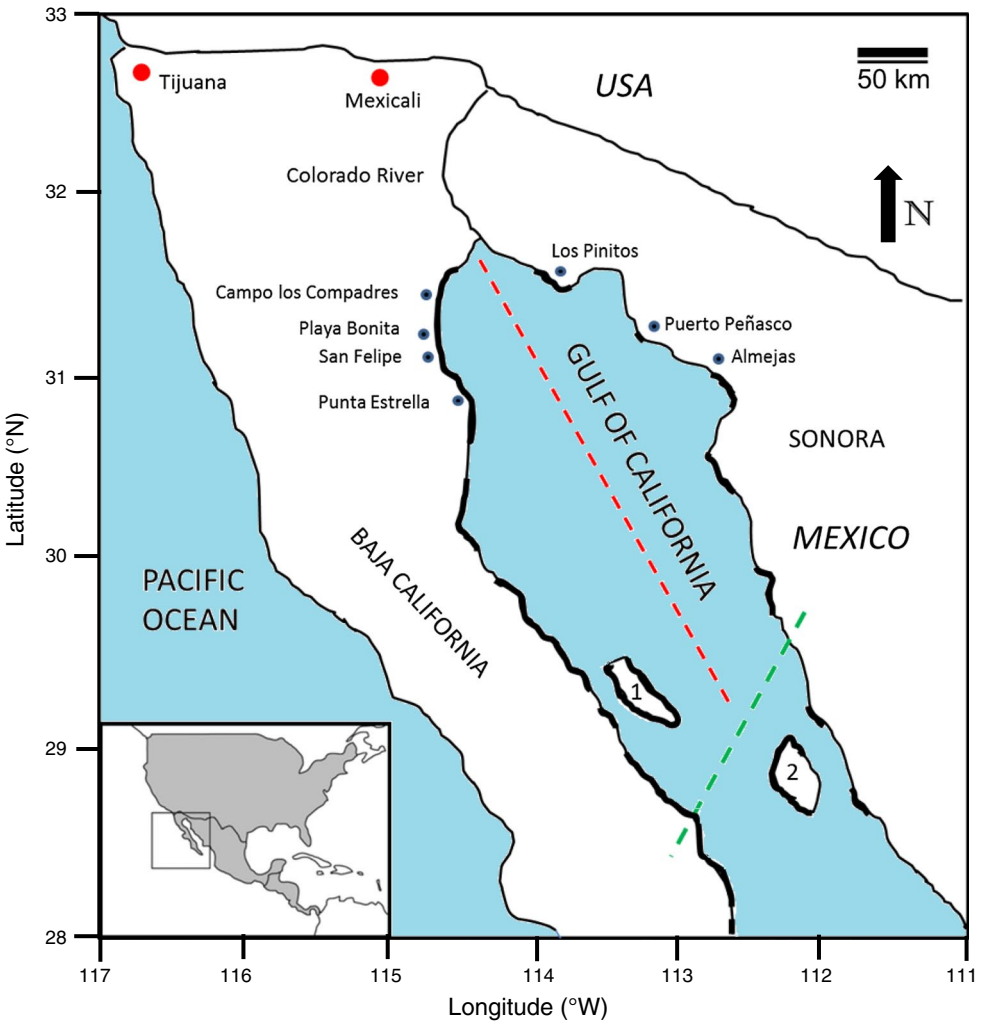

Fig. 1 Map of the NGC. *Thick coastlines* represent rocky shore and sampling locations are indicated by *blue circles*. Biogeographic regions are separated by the *dotted lines*: *red line* boundary between the east and west coasts, *green line* boundary between the northern and central Gulf regions. Islands within the Gulf are as follows: (1) Isla Angel de la Guarda and (2) Isla Tiburon. Redrawn from Riginos and Nachman (2001)

contradiction: *Littorina saxatilis*, a brooding gastropod (as well as several other invertebrate species lacking a planktonic stage) has successfully colonized isolated island sites, whereas *Littorina littorea*, with planktotrophic larvae, has not (Johannesson 1988).

These inconsistencies highlight the fact that dispersal and gene flow in marine systems often reflect complex interactions involving multiple life-history traits and larval behaviors, patterns of water circulation, historical and contemporary barriers to dispersal, and the spatial distribution of suitable habitat (Perrin and Borsa 2001; Marko 2004; Lourie et al. 2005; Ayre et al. 2009; Cowen and Sponaugle 2009; Morgan et al. 2009; Pinsky et al. 2012). For example, dispersal by gametes (Grosberg 1991; Yund 1995; Bishop 1998), detached larvae (Martel 1993), and adults (Hellberg 1994, 1996; Worcester 1994), may lead to more extensive gene flow and larger ranges than would be expected based on larval dispersal potential alone (Winston 2012). Disentangling the contributions of each of these processes to contemporary genetic structure is essential for understanding present-day dispersal routes, scales of connectivity, and population dynamics.

The northern Gulf of California (NGC) is a region of primary importance to Mexican fisheries and has been identified as a conservation priority, currently encompassing three marine protected areas (MPAs), including two biosphere reserves (Cisneros-Mata 2004; IUCN 2005). The NGC has a complex geologic and oceanographic history and is one of the most productive and diverse marine ecosystems in the world (Ezcurra et al. 2002). Its waters exhibit extreme seasonal temperature variation, tidal amplitudes that may exceed 10 m, and a diversity of substrates and habitats (Briggs 1974). The Baja California coast of the NGC consists largely of rocky shores interspersed with short stretches of sand and sandy mud (Fig. 1). All of the mainland coastline of the NGC lies within the state of Sonora. The intertidal and shallow subtidal zones consist of sand and mud forming long beaches and estuaries, only occasionally interrupted by rocky outcrops and boulder fields (Fig. 1; Brusca 2004). In addition, the Colorado

River forms a large delta, emptying into the NGC between Baja California and Sonora, potentially creating a boundary between the two coasts.

Despite the biological and economic importance of the NGC, little is known about population connectivity and dispersal patterns for most of its resident species. Previous studies of Gulf fauna have typically concerned regional scale patterns (>100–1,000 km) of genetic differentiation (e.g., Riginos and Nachman 2001; Bernardi et al. 2003; Riginos 2005; Hurtado et al. 2007; Deng and Hazel 2010), focusing less on the effects of smaller-scale oceanographic or habitat features on population genetic structure. Organisms restricted to patchy habitats and with low dispersal potential represent promising systems in which to study fine-scale patterns and processes of divergence. On the one hand, high levels of population subdivision are expected in animals restricted to a single patchy habitat type, because surrounding unsuitable habitats can constitute effective dispersal barriers (Hurtado et al. 2010). Gene flow should be limited to proximate populations, with genetic differentiation increasing between increasingly distant populations (i.e, isolation-by-distance; Wright 1943). On the other hand, whether limited larval dispersal translates into a geographical pattern of gene flow consistent with an isolation-by-distance model remains unclear because mechanisms other than the movement of larvae may influence population connectivity in marine species (Hellberg 1994).

In this paper, we characterize patterns of genetic structure in the buccinid whelk, *S. macrospira,* a gonochoric, direct-developing intertidal gastropod which produces benthic egg capsules and is restricted to rocky outcrops on mudflats in the upper NGC. Its widespread distribution on both coasts of the upper Gulf coupled with its habitat specificity presents an opportunity to simultaneously evaluate the importance of several potential mechanisms on patterns of population differentiation. Specifically, we test these hypotheses relating to patterns of genetic structure in *S. macrospira*: (1) The open waters of the NGC present a significant barrier to dispersal, leading to deep intraspecific divergence between Sonoran and Baja Californian populations; (2) direct development and limited dispersal should lead to a strong positive relationship between geographical and genetic distances between populations; and (3) populations separated by unsuitable habitat (sandy substrate) should be more differentiated than populations separated by suitable habitat (rocks).

We use three molecular markers (nDNA, mtDNA, and microsatellites) to infer patterns of divergence among populations across most of the documented range of *S. macrospira*. We utilize this approach because the most complete explanation for both past and present levels of population connectivity can only be achieved by exploiting the variable mutation rates present among the different markers. For example, rapidly evolving loci such as microsatellites are suited to measuring contemporary gene flow, whereas slower evolving markers, like mtDNA, reveal patterns of divergence that have accrued over hundreds to thousands of generations (Wares and Cunningham 2001; Marko and Hart 2011; Doellman et al. 2011; Panova et al. 2011). Past events can thus be examined over wider time scales, allowing for the separation of different aspects of a species' evolutionary history (Crandall et al. 2000; Sunnucks 2000; Garrick et al. 2009). We conclude by assessing whether the relative importance of the mechanisms outlined above (i.e., open water, geographical distance, and discontinuities in suitable habitat) varies across historical and contemporary time scales. Population genetic patterns offer clues to past geological and environmental events, which, in turn, can improve our understanding of the complex relationships among life histories, biogeography, oceanography, and genetic structure in the northern Gulf region.

Materials and methods

Specimen collection and sampling

Solenosteira macrospira is an intertidal buccinid whelk, native to the NGC, always found associated with rocks and boulders on mudflats and sandy shores. Its range extends south to Puertecitos (30°35.06″N, 114°63.89″W) on the Baja California side, and to Guaymas (27°91.83″N, 110°89.89″W) on the Sonoran side (Brusca 2004; Houston 1978). During the spawning season (March–May), females attach egg capsules to the shells of male conspecifics (Kamel and Grosberg 2012). Each egg capsule contains ≈200–300 eggs; on average, 3–20 hatchlings per capsule emerge as crawl-away juveniles, about 1 month after oviposition. We sampled a total of 588 individual *S. macrospira* from seven locations in the upper NGC (Fig. 1). We collected snails from the Puerto Peñasco (Sonora) ($n = 144$) and San Felipe (Baja California) ($n = 139$) sites in April 2004, and again from Puerto Peñasco ($n = 143$) in April 2005, as part of another project. In May 2007, we collected individuals from all other locations ($n = 19$–38 snails per site). Upon collection, we removed the snails from their shells and preserved them in 95 % ethanol.

Genetic data collection

Mitochondrial and nuclear genes

We extracted genomic DNA from ethanol preserved muscle tissue following the cetyltrimethyl ammonium bromide (CTAB) protocol described in Grosberg et al. (1996). We used PCR to amplify fragments of two mitochondrial

genes (cytochrome *c* oxidase subunit I, *COI*; cytochrome b, *Cyt b*), and an intron from the single-copy nuclear gene *Calmodulin* (*Cal*). We used the universal primers LCO1490 and HCO2198 to amplify COI (Folmer et al. 1994), and we used the complete mtDNA genome of *Ilyanassa* (=*Nassarius obsoleta*) to design primers to amplify 880 base pairs of *Cyt b* (Io7908L 5′- WCACTATACKG CHCATGTAG-3′ and Io8794R 5′-TGYTCATAHGGWG YTTCWACVGG-3′). We identified the nuclear *cal* gene through BLAST searches of *I. obsoleta* expressed sequence tags (ESTs) deposited in GenBank (GenBank accession no. EV826015). Using custom primers anchored in the coding region (IoCalL 5′-CTGAGATCAAGGAGGCGTTG-3′ and IoCalR 5′-CACCATCAAGGTCAGCC TCT-3′), we identified and sequenced a combination of coding and non-coding (intron) DNA. From these preliminary sequences, we designed species-specific exon-primed intron crossing (EPIC) primers for the *cal* gene in *Solenosteira* (SmCalL 5′-ATCCATCTGTGCCAGA AACC-3′; SmCalR 5′-AACAGCCCTCAACACAGCTT-3′).

We performed all PCRs in 15 µL volumes using an Applied Biosystem GenAmp®9600 thermal cycler. PCR mixes consisted of 15–30 ng of template DNA, 1× PCR Buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP (Promega), 0.1 mg/mL bovine serum albumin (BSA, NEB), 0.25 µM forward and reverse primers, 0.6 U of AmpliTaq® DNA polymerase (Applied Biosystems). The cycling protocol consisted of an initial denaturation step of 3 min at 94 °C, followed by 35 cycles of 94 °C denaturation for 30 s, annealing at 49 °C for 30 s, and extension at 72 °C for 50 s for *COI* and *Cytb*, and a 94 °C denaturation for 30 s, annealing at 56 °C for 30 s, and extension at 70 °C for 1 min for *cal*. All PCR protocols included a final extension step at 72 °C for 3 min. We treated PCR products with Exonuclease 1 (USB) and Shrimp Alkaline Phosphatase (USB) and direct sequenced with one or both primers using an Applied Biosystems 3730 Capillary Electrophoresis Genetic Analyzer. Sequences were edited and aligned using the SEQUENCE NAVIGATOR and AUTO ASSEMBLER programs (Applied Biosystems). We inferred the allelic phases of individuals heterozygous at the *cal* gene using the program PHASE version 2.1.1 (Stephens et al. 2001; Stephens and Scheet 2005). This program uses a Bayesian method to reconstruct haplotypes from a population sample and to estimate the uncertainty associated with each phase call. We ran the program using the default model allowing for recombination (-MR0 flag) for 1,000 iterations with a burn-in of 1,000 and a thinning interval of five. We repeated the analysis five times and examined the haplotype frequency estimates and goodness-of-fit measures to ensure consistency across runs. We excluded individuals whose haplotype reconstructions were ambiguous ($P < 0.95$) from the data analysis. All sequences were deposited in GenBank (Accession numbers KF956938-KF957436).

Microsatellites

Ecogenics GmbH (Zurich, Switzerland) constructed a library from size-selected genomic DNA ligated into SAULA/SAULB-linker (Armour et al. 1994) and enriched by magnetic bead selection with biotin-labeled $(AAG)_{10}$ and $(GCGT)_7$ oligonucleotide repeats (Gautschi et al. 2000a, b). The microsatellite PCR amplification mixture contained 15–30 ng of template DNA, 1× PCR Buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP (Promega), 0.1 mg/mL bovine serum albumin (BSA, NEB), 0.25 µM forward and reverse primers (Table S1), 0.6 U of AmpliTaq® DNA polymerase (Applied Biosystems). The cycling protocol consisted of an initial denaturation step of 3 min at 94 °C, followed by 35 cycles of 94 °C denaturation for 30 s, annealing at 49 °C for 30 s, and extension at 72 °C for 30 s. One microliter of the amplification products was added to 9 µL of formamide and ABI Gene Scan 500 size standard and loaded onto a 96-well plate for analysis on an ABI Prism 3100 Capillary Electrophoresis Genetic Analyzer. We visualized and scored fragment data using STRand (Version 2.3.69; Toonen and Hughes 2001).

Analyses

Genetic diversity

For the microsatellite loci, we used GENEPOP 3.4 (Raymond and Rousset 1995) to estimate expected heterozygosity (H_E) and to test for linkage disequilibrium and conformity to Hardy–Weinberg expectations (HWE). We calculated allelic richness (R_S) using FSTAT 2.9.3.2 (Goudet 1995), by standardizing to the smallest sample size ($n = 19$). For the DNA sequence data, we used DNASP 4.90 (Rozas et al. 2003) to calculate haplotype diversity (h), and nucleotide diversity (π).

Genetic differentiation

We calculated Weir and Cockerham's F statistics (F_{IS}, F_{IT}, F_{ST}) for both the microsatellite markers and DNA sequences using ARLEQUIN (Excoffier et al. 2005) and tested for significance by 10,100 permutations of the data. We tested for pairwise differences between populations using FSTAT (Goudet 1995) for the microsatellites and ARLEQUIN for the DNA sequences.

Open water as a barrier to gene flow

We used an analysis of molecular variance (AMOVA) to test whether there was regional genetic subdivision between the east and west coasts of the Gulf. We ran the AMOVAs using F statistics for the microsatellites and Tamura and

Nei (1993) distances for the DNA sequences and tested for significance with 10,100 permutations of the data. To visualize the phylogenetic relationships among mtDNA and *cal* sequences, we constructed 95 % statistical parsimony networks using TCS 1.18 (Clement et al. 2000).

We used the Bayesian model-based clustering algorithm implemented by the program structure 2.2.3 (Pritchard et al. 2000) to characterize the underlying genetic structure in the microsatellite data. We assessed the number of genetic clusters (K) among our seven *S. macrospira* samples for values of K ranging from 1 to 10 using the admixture model, with allele frequencies correlated among populations and ignoring prior population information. We ran 10 Bayesian Markov Chain Monte Carlo (MCMC) searches of between 150,000 and 1,000,000 steps with a burn-in of 10 %, and used the method of (Evanno et al. 2005) to find the best-fit value of K.

To estimate the magnitude of gene flow (m) and divergence times (t) between populations on the east and west sides of the NGC, we ran a coalescent-based analysis of the *COI* data in IMa2 (Hey and Nielsen 2007), with a MCMC approach under an isolation with migration (IM) model of divergence. We conducted preliminary runs using very large priors to determine the maximum values of the model parameter estimates. Our final analyses were run with a burn-in until a flat trend-line appeared, after which we sampled 200,000 genealogies. We used a conservative mutation rate for *COI* of 2.3 % per million years (Knowlton et al. 1993; Hellberg and Vacquier 1999).

Dispersal routes and isolation-by-distance in S. macrospira

Since little is known about dispersal routes in the NGC, especially for species that lack pelagic larvae, we investigated several dispersal scenarios for *S. macrospira*. We constructed our geographical distance matrices such that sampled populations were joined in a stepping-stone manner, and we tested four connectivity hypotheses. Geographical distances between populations were measured using several dispersal routes: (1) an inverted U pattern, assuming no dispersal across the southern waters of the NGC; (2) a U-shaped pattern, assuming dispersal across the southern waters of the NGC; (3) the shortest shoreline distance from either of the above directions; or (4) great circle distances (i.e., the shortest distances between points along the surface of the Earth). To characterize the relationship between inferred levels of gene flow and geographical distance between populations, we conducted Mantel tests for nonrandom associations between matrices of geographical and genetic distances using the program IBDWS (Jensen et al. 2005). We calculated genetic distances using Slatkin's (1993) similarity index, $\widehat{M}$, based on allelic frequencies for the microsatellites and Kimura 2-parameter distances for the DNA sequences. We used an AIC model selection procedure to identify which of the geographical distance matrices best explained the patterns of genetic distance (Burnham and Anderson 2002).

Open water, distance, and unsuitable habitat as barriers to gene flow

Several factors, acting singly or in combination, could account for the observed patterns of genetic differentiation in *S. macrospira*. To explore the collective effects of open water, geographical distance and the distribution of suitable habitat on genetic distance, we conducted multiple regression analyses on all markers. First, we defined three prediction vectors reflecting distances between population pairs based on (a) open water (0 = same coast, 1 = different coast; WATER); (b) geographical distance (km separating the populations; SDIST); (c) discontinuous habitat due to sandy shores (0 = population pairs separated by rocky shore or open water, 1 = population pairs separated by sandy shore; SAND). We used the geographical distance vector corresponding to the U-shaped pattern with dispersal across the south NGC (SDIST), since this matrix had the lowest AIC value (see "Results"). Although there were three types of habitats separating the population pairs (water, sand, and rocks), the effects of these habitats could not be estimated independently but could only be estimated relative to each other. This is because assigning three presence/absence binary codes for each habitat type resulted in a design matrix that was not invertible and could not be solved for a least squares solution. Therefore, the predictor variables WATER and SAND represent the effect of open water minus the effect of rocky shore and the effect of sandy shore minus the effect of rocky shore, respectively. This makes it possible to test whether discontinuous habitat (water or sand) increases genetic distance relative to continuous habitat (rock). In both cases, a positive correlation coefficient would indicate that the populations separated by discontinuous habitat are more genetically distant than populations separated by continuous habitat (see Riginos and Nachman (2001) for a similar approach). We standardized all vectors by subtracting the mean and dividing by one SD (Selkoe et al. 2010). The sums of squares and associated statistics for the whole model were calculated following standard regression procedures. We estimated significances for the whole model and partial regression coefficients by permuting the order of values in the vector of genetic distances and recalculating the regression 10,000 times using the program RT version 2.1 (Manly 1997).

Results

Genetic diversity

We genotyped 588 specimens of *S. macrospira* from seven locations in the upper NGC. Our sequence alignments

Table 1 Summary of sample sizes and genetic diversity for the microsatellites, the combined mtDNA sequences (*COI* + *CytB*), and the *Calmodulin* intron

Sample site	mtDNA—1,390 bp					*Calmodulin*—557 bp				Microsatellites—6 loci			
	N	n_H	*h*	H_d	π	n_D	*h*	H_d	π	n_A	*Ho*	*He*	R_s
West NGC													
Punta Estrella (PE)	38	25	6	0.4267	0.0003	46	3	0.4050	0.0020	38	0.58	0.69	7.3
San Felipe (SF)	138	26	10	0.7385	0.0008	58	2	0.1000	0.0005	138	0.67	0.69	6.5
Playa Bonita (PB)	33	20	8	0.7000	0.0007	42	1	0.0000	0.0000	33	0.65	0.71	7.4
Campo los Compadres (CC)	37	21	6	0.5571	0.0007	40	7	0.4590	0.0027	37	0.68	0.78	8.4
East NGC													
Los Pinitos (LP)	19	19	5	0.7310	0.0010	34	5	0.8220	0.0036	19	0.73	0.73	5.6
Puerto Penasco (PP)	287	29	5	0.7414	0.0012	60	6	0.6430	0.0027	287	0.71	0.72	6.2
Almejas (AS)	36	23	10	0.6403	0.0011	32	6	0.5080	0.0026	36	0.63	0.76	6.2
Total	588	163	38	0.7139	0.0010	312	14	0.5720	0.0029	588	0.66	0.73	6.8

n_H number of haplotypes sampled, n_A number of alleles sampled, n_D number of diploid genotypes, H_O observed heterozygosity, H_E expected heterozygosity, R_S average allelic richness, *h* number of unique haplotypes, H_d haplotype diversity, π nucleotide diversity

included 609 bp of *COI*, 781 bp of *CytB*, and 557 bp of the nuclear intron *Cal*. We detected 13 unique haplotypes for *COI* and 32 haplotypes for *CytB*; nucleotide polymorphism (π) was more than twice as high for *CytB* (0.0013) than for *COI* (0.0005). Since many populations of *S. macrospira* had only one or two unique *COI* haplotypes, we combined both *COI* and *CytB* into a single 1,390-bp haplotype in the individuals for which we sequenced both fragments ($n_H = 163$; $h = 38$; Table 1).

We reconstructed the nuclear haplotypes using the maximum-likelihood methods implemented by PHASE, which successfully identified allelic states with >95 % certainty in 156 of the 163 (>96 %) individuals we directly sequenced for the *Cal* intron. We detected 14 unique haplotypes, and haplotype diversity (H_d) ranged considerably from 0 at Playa Bonita (PB) in the western gulf (Baja California) to 0.822 at Los Pinitos (LP) on the eastern shore of Sonora (Table 1).

We obtained multi-locus microsatellite genotypes for 588 individuals. Observed heterozygosities ranged from 0.58 to 0.73 among all seven *S. macrospira* populations (Table 1), with between 7 and 16 alleles per locus averaged across seven populations (Table S1). We detected a small but significant excess of homozygotes at three loci (*Soleno01*, *Soleno13*, *Soleno22*), and the exact test of Raymond and Rousset (1995) indicated a small but significant global departure from HWE ($F_{IS} = 0.014$, $P < 0.001$, Table S1). There was no signal of linkage disequilibrium between microsatellite loci (data not shown).

Open water as a barrier to gene flow

The AMOVA analysis, comparing eastern versus western populations from the NGC, revealed a strong and significant hierarchical structure for the mtDNA and microsatellite loci, but not the *Cal* intron (Table 2). With the exception of the microsatellites, for which pairwise differences among populations were less variable than those using mtDNA, the proportion of genetic variation among populations within groups was very high. This suggests that the non-significant among-group comparison for *Cal* might be due to the exceptionally high pairwise differentiation between populations (Table 2).

Parsimony networks constructed with both the mtDNA and *Cal* DNA sequences revealed striking differences in the frequency of unique haplotypes, supporting genetic structuring across the Gulf (Fig. 2). Both genes had common and widespread haplotypes found at high frequency in all populations, but they also had several low frequency haplotypes unique to individual locations on both the eastern and western coasts of the Gulf.

Bayesian clustering analyses identified two significant clusters of microsatellite genotypes which corresponded to the four populations sampled from the western NGC and the three populations sampled from the eastern NGC. Although the variance in the posterior probability of the estimate of the number of clusters in the microsatellite data was high for values of $k \geq 4$, the Δk method of Evanno et al. (2005) showed a strong mode at $k = 2$ that was more than 6× higher than at $k = 3$.

Overall, our data support a strong east–west structuring of *S. macrospira* in the NGC. We therefore used IMa2 to infer *t*, the time since the populations on both sides of the Gulf diverged. Using a divergence rate of 2.3 % mya for the *COI* data, our multi-locus mtDNA analysis suggests that the eastern and western Gulf populations split approximately 22,071 years ago (95 % CI 8,614–58,157).

Table 2 *Solenosteira macrospira* population structure from the analysis of molecular variance (AMOVA) comparing the population groups along the western (PE, SF, PB, CC) and eastern (AS, PP, LP) coasts of the NGC

Source of variation	Variance components	Percentage of variation
mtDNA (*COI* + *CytB*)		
Among groups (Φ_{CT})	0.07**	7.11
Populations within groups (Φ_{SC})	0.126**	11.67
Within populations (Φ_{IS})	0.188**	81.22
Φ_{ST}	0.163**	
Cal		
Among groups (Φ_{CT})	0.139	13.87
Populations within groups (Φ_{SC})	0.304**	26.27
Within populations (Φ_{IS})	0.401**	59.86
Φ_{ST}	0.362**	
Microsatellites		
Among groups (Φ_{CT})	0.052*	5.15
Populations within groups (Φ_{SC})	0.048**	4.56
Within populations (Φ_{IS})	0.097**	90.30
Φ_{ST}	0.083**	

Φ_{ST} represents the magnitude of genetic subdivision among populations in the absence of hierarchical structure

* $P < 0.01$; ** $P < 0.001$

Dispersal routes and isolation-by-distance in *S. macrospira*

Since little is known about dispersal routes around the NGC, we used an isolation-by-distance model to explore several scenarios in *S. macrospira*. Across all three marker classes, the AIC scores were lowest for the models with the U-shaped southern dispersal route as the predictor of genetic distances (Table 3), suggesting this is the most likely dispersal route connecting the east (Sonora) and west (Baja California peninsular) coasts. The geographical matrix based on this dispersal route explained 48 % of the variation in the mtDNA divergence ($P = 0.004$) and 26 % of the divergence at the microsatellite loci ($P = 0.020$). The results for all other hypothesized routes were not significant. Significant values are indicated in bold.

Open water, distance, and unsuitable habitat as barriers to gene flow

The multiple regressions of the three predictor vectors on genetic distance were highly significant, but differed across each of the three marker classes (Table 4). For the mtDNA, the partial regression coefficient of SDIST (i.e., U-shaped dispersal across the southern part of the upper NGC) was significantly greater than random (whole model: $r^2 = 0.89$, $F_{1, 20} = 34.84$, $P < 0.001$). For the *Cal* intron, the partial regression coefficients of WATER and SAND explained a significant proportion of the variance in genetic distance (whole model: $r^2 = 0.39$, $F_{1, 20} = 3.92$, $P = 0.047$). The significant positive coefficient for WATER suggests that populations separated by water are more distantly related to each other than populations separated by either rocks or sand. However, the significant negative coefficient for SAND indicates that populations separated by sand are, on average,

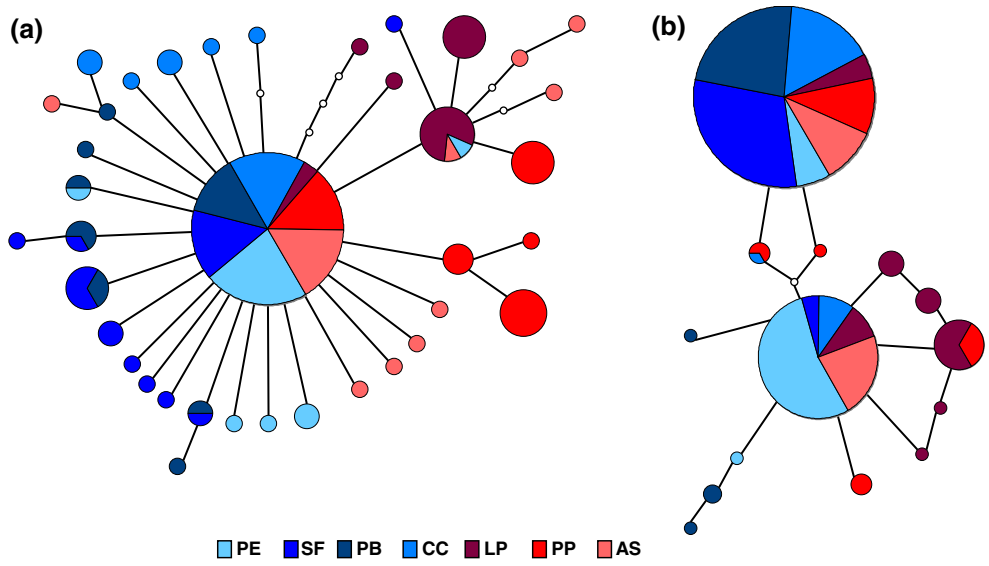

Fig. 2 95 % plausible networks for **a** *S. macrospira* mtDNA (*COI* + *CytB* $n = 163$) and **b** *S. macrospira* ($n = 312$) *cal* intron haplotypes from the NGC. Diameters of circles are proportional to the frequency of the haplotype, lines connect haplotypes differing by one mutation, and *small open circles* indicate missing intermediate haplotypes. Locations sampled from the western NGC are shades of *blue* and locations from the eastern NGC are shades of *red*. Sampling abbreviations are as in Table 1

Table 3 Isolation by distance tests for correlations between genetic differentiation (F_{ST}) and geographical distance between sampling locations

Marker	Distance measurement	AIC	R^2	P value
mtDNA	(a) Inverted U dispersal	−19.03	0.02	0.22
	(b) U-shaped dispersal	**−32.27**	**0.48**	**0.004**
	(c) Shortest shoreline route	−19.96	0.06	0.13
	(d) Great circle distance	−18.69	0.001	0.32
Cal	(a) Inverted U dispersal	0.09	0.01	0.54
	(b) U-shaped dispersal	**−0.23**	**0.02**	0.15
	(c) Shortest shoreline route	0.13	0.004	0.53
	(d) Great circle distance	0.05	0.01	0.71
Microsatellites	(a) Inverted U dispersal	−80.33	0.19	0.07
	(b) U-shaped dispersal	**−82.52**	**0.26**	**0.02**
	(c) Shortest shoreline route	−80.45	0.19	0.06
	(d) Great circle distance	−78.35	0.11	0.10

Mantel tests (10,000 permutations) were conducted on distance matrices that correspond to four different dispersal scenarios (see text)

Table 4 Partial regression coefficients of biogeography, geographical distance, and habitat for a multiple linear regression on genetic distance with probabilities determined by a modified Mantel test

Marker	Variable	Partial regression coefficient (β)	t value	P value	R^2
mtDNA	WATER	0.13	0.89	0.34	
	SAND	0.04	0.27	0.79	
	SDIST	0.21	**3.96**	**0.001**	0.89
Cal	WATER	1.42	**2.27**	**0.04**	
	SAND	−1.44	**−2.24**	**0.04**	
	SDIST	0.11	0.48	0.64	0.59
Microsatellites	WATER	0.24	**3.38**	**0.004**	
	SAND	−0.25	**−3.48**	**0.003**	
	SDIST	0.03	0.96	0.35	0.71

Significant values are indicated in bold

genetically more similar than populations separated by rocks. The microsatellite data display a pattern consistent with the *Cal* intron: the multiple regression explained 71 % of the variation ($r^2 = 0.71$, $F_{1,\ 20} = 7.14$, $P = 0.001$), and the partial regression coefficients of WATER and SAND significantly differed from zero ($P < 0.05$).

Discussion

As expected for a species with direct development and limited mobility of adults, we found substantial population structure at both local (<20 km) and regional (~400 km) scales in populations of *S. macrospira* sampled from seven sites across its range in the upper NGC. However, like in many other species with limited dispersal potential (reviewed in Hellberg 2009), patterns of genetic structure did not conform to a simple linear stepping-stone model, where the magnitude of genetic differentiation was a monotonic function of geographical distance between sampled populations. Instead, multiple factors, some historical, some contemporary, appear to govern the present-day distribution of genetic variation.

Open water as a barrier to gene flow

At the regional scale, phylogenetic relationships among mtDNA haplotypes, AMOVA analyses, and Bayesian assignment tests all show a significant break between populations in the eastern and western NGC. The demographic reconstruction suggests that the split between eastern and western populations occurred approximately 20,000 years ago, coinciding with a rise in sea levels in excess of 20 m after the last glacial maximum (26,500–20,000 years ago) (Fairbanks 1989; Weaver et al. 2003).

Dispersal routes and isolation-by-distance in *S. macrospira*

At a more local scale, a U-shaped southern dispersal route best explains the relationship between genetic distance and geographical distance. This pattern of isolation-by-distance is evident from both mtDNA and microsatellite markers, suggesting that while the open waters of the NGC prevent extensive mixing between eastern and western populations, snails have been able to traverse the southern part of the NGC over both historical and contemporary time scales, encompassing periods when sea levels were both low and high.

How did *S. macrospira* successfully disperse across the waters of the NGC? Given that the upper NGC is flat and shallow, it likely contracted significantly during the low sea level periods of the Pleistocene (Hurtado et al. 2010), potentially enabling stepping-stone dispersal across rocky outcrops along the southern portion of *S. macrospira*'s range (e.g., Hellberg 1995). However, the southern dispersal route is also the best-fit model for the genetic distances reflected by the microsatellite loci, which—given their rapid rates of evolution—most directly represent contemporary gene flow. Given the post-Pleistocene rises in sea level that occurred in the Gulf, with maximal heights estimated to be about 4–9 m above present levels (Ortlieb 1981, 1984), this pattern of contemporary connectivity might best be explained by the presence of a strong cyclonic (e.g., anti-clockwise) gyre present in

the NGC that could transport rafted adults and juveniles across the open waters in the southern part of their range (Marinone et al. 2008; see section below). In this case, egg-carrying males that are advected to suitable habitats and release their offspring could be successful colonizers of new environments (Thiel and Haye 2006), in part because offspring will hatch within a restricted area and potentially remain there until sexually mature, increasing the encounter rates of mates (Johannesson 1988; Schulze et al. 2000).

The isolation-by-distance analyses also confirm that the Colorado River delta represents a barrier to dispersal in *S. macrospira*, since none of the models of dispersal which included movement across it were significant. This result is mirrored in the patterns of genetic differentiation among populations: the two populations on either side of the delta [(i.e., CC and LP) were highly significantly differentiated across all markers; Table S2].

Open water, distance, and unsuitable habitat

When considering all variables simultaneously, mtDNA markers revealed that geographical distance (based on a southern dispersal route: SDIST) was the only significant factor influencing patterns of population structure during the Pleistocene (ca. 125 kya), suggesting that *S. macrospira* could disperse across the southern portion of the upper NGC. Dispersal was likely facilitated by the low sea level periods of the Pleistocene (Hurtado et al. 2010). On the other hand, the *Cal* intron and the microsatellites, reflecting more recent population processes, show that the open waters of the NGC (WATER) now play a dominant role in structuring populations and that geographical distance does not contribute significantly to explaining patterns of contemporary population structure.

Contrary to expectations, unsuitable habitat (SAND) did not present a significant barrier to gene flow: both nuclear markers indicated that populations separated by sand were more genetically similar than populations separated by rocks. This is surprising given that areas lacking rocky shore are typically strong barriers to gene flow in species restricted to rocky intertidal habitats (Billot et al. 2003; Johansson et al. 2008; Ayre et al. 2009). However, Johannesson and Warmoes (1990) found that the direct-developing gastropod *L. saxatilis* colonized breakwaters along the Belgian coast that were often separated by kilometers of unsuitable sandy habitats. They hypothesized that strong currents running parallel to the shoreline transported snails between breakwaters. Recent studies of other benthic invertebrates with direct development also suggest that long-distance dispersal via rafted transport on macroalgal mats may extend connectivity beyond that expected from pelagic larval duration alone (Helmuth et al. 1994; Waters and Roy 2004; Donald et al. 2005; Thiel and Haye 2006; Miranda and Thiel 2008).

Moreover, studies of connectivity in the NGC using numerical particle tracking have shown that strong regional hydrodynamics produce a basin-wide cyclonic (anti-clockwise) gyre, which lasts throughout the summer months, from June to August (Carrillo-Bribiezca et al. 2002; Marinone et al. 2008, 2011). Flow patterns are strongly northward on the eastern Sonoran coast but weakly southward on the peninsular side. Density distributions of juvenile rock scallops (*Spondylus calcifer*) and black murex (*Hexaplex nigritus*), species whose larvae are considered passive, are consistent with these flow patterns (Cudney-Bueno et al. 2009). Larval transport by this cyclonic gyre has also been documented in larvae of the shrimp species *Litopenaeus stylirostris* and *Farfantepenaeus californiensis*, and the main pathway of post-larval shrimp to the Colorado River delta is via the Sonoran shoreline (Galindo-Becti et al. 2010). On the western coast of the NGC, circulation patterns are significantly weaker and numerical simulations reveal that particles do not travel far from their origin in this region of the NGC (Marinone et al. 2011). Thus, the combination of limited dispersal and weak circulation patterns on the west coast could explain the higher level of population structure (i.e., populations less than 20 km apart are significantly more differentiated than their counterparts on the east coast), despite the presence of largely continuous rocky habitat. Though adult *S. macrospira* are strongly associated with intertidal rocky substrate during the reproductive season, they may migrate offshore into deeper waters outside of the mating season. Given that sandy mud and deeper waters have been minimally sampled for this species, it may well be that rafting or currents transport adult snails substantial distances across unsuitable reproductive habitat. To our knowledge, there are no obvious barriers preventing *S. macrospira* from crossing one rocky area to the next, and so it remains an open question as to why this pattern of differentiation exists.

Conclusions

Our range-wide study of genetic structure in *S. macrospira* in the NGC suggests that on contemporary time scales physical transport processes strongly influence local and regional genetic differentiation, even in a species with direct benthic development. This underscores the strong, and potentially homogenizing, effect of present-day ocean circulation patterns in the NGC (Cudney-Bueno et al. 2009; Aznar et al. 2010; Galindo-Becti et al. 2010). On longer temporal and broader spatial scales, glacially driven marine transgressions and regressions also appear to leave

signatures on structure, evident in more slowly evolving genetic markers. Given our meager understanding for most species of the complex interactions among developmental mode, habitat distribution, and circulation that determine genetic structure, it remains a major challenge to understand patterns of connectivity in this ecologically and economically important region.

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